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ACTIVATED T-CELLS AND THEIR USES

1. FIELD OF THE INVENTION

The present invention relates to compositions and methods for the treatment or diagnosis of injury of the central nervous system (CNS). In an embodiment, activated T-cells are used to deliver (a) a diagnostic substance for detecting a site of injury or disease or (b) a therapeutic substance for ameliorating an effect of a disease or injury, such as, for e.g., promoting axonal regeneration or preventing or inhibiting degeneration caused by injury or disease. In a preferred embodiment, the activated T-cells that are used for delivering a substance do not recognize a nervous system (NS) antigen. More preferably, the substance-delivering activated T-cells recognize a non-self antigen (*e.g.*, ovalbumin). In another embodiment, pharmaceutical compositions comprising antiself T-cells that recognize an antigen present in a greater concentration in the nervous system (NS) compared to the other organs or circulation are used to prevent or inhibit degeneration of nerves within the CNS. In a preferred embodiment the antiself T-cells of the present invention are not genetically-engineered. In another embodiment, pharmaceutical compositions comprising an antigen (or derivative thereof) present in a greater concentration in the NS compared to other organs or the circulation (NS-specific antigen or derivative) are used to prevent or inhibit degeneration of nerves within the CNS. The activated T-cell compositions of the present invention may be administered alone or in combination with NS-specific antiself T-cells or NS-specific antigen or in combination with NS-specific antiself T-cells and NS-specific antigen.

2. BACKGROUND OF THE INVENTION

Damage to the CNS may result from a traumatic injury, such as penetrating trauma or blunt trauma, or a disease or disorder, including but not limited to Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease, amyotrophic lateral sclerosis (ALS) and ischemias.

Following traumatic injuries in the peripheral nervous system (PNS), an invasion of blood derived monocytes as well as activation of microglia within the PNS occurs (Stoll, et al., 1989, *Neurosci.*, 9:2327-35; Perry and Gordon, 1991, *Int. Rev. Cytol*, 125:203-44; Perry and Gordon, 1988, *Trends Neurosci.*, 11:273-277; Jordan and Thomas, 1988, Brain macrophages: *Questions or origin and Interrelationships*, 13:165-178; Griffin, et al., 1990, *Ann. Neurol.*, 27:8; Giulian, et al., 1989, *J. Neurosci.*, 9:4416-29; Giulian, 1987, *J. Neurosci. Res.*, 18:155-171; de Groot, et al., 1989, 179:314-27; and Bauer, et al., *J. Neurosci. Res.*, 38:365-75). By contrast, the invasion of blood derived monocytes is delayed and more limited in its scope in traumatic injuries to the CNS (Perry and Gordon, 1991, *Int. Rev. Cytol*, 125:203-44; Andersson, et al., 1991, *Immunol. Lett.*, 30:177-81; and Perry et al., 1987, *J. Exp. Med.*, 165:1218-1223). In addition, the duration of events associated with the acute phase of the injury, though less pronounced, is prolonged in the CNS as compared to the PNS. For example, several weeks after injury, numerous activated macrophages and microglia are found in the CNS, while only a few are detectable in PNS nerves at such time after injury (Perry et al., 1987, *J. Exp. Med.*, 165:1218-1223; Lunn *et al.*, 1990, Neuroscience 35:157-165).

Neurons in mammalian CNS do not undergo spontaneous regeneration following an injury. Thus, a CNS injury causes permanent impairment of motor and sensory functions. In contrast, neurons in the PNS have a far greater capacity to regenerate. Studies using allogenic macrophages incubated with a stimulant (*e.g.* a nerve segment) and subsequently administered into the CNS of a mammal at or near the site of injury have shown regeneration of the impaired motor or sensory function (PCT Publication WO 97/09885 and Spiegler *et al.*, 1996, FASEB J. 10:1296).

Another tragic consequence of CNS injury is that the primary injury is often compounded by a degenerative process which results in a secondary loss, over time, of adjacent

neurons that were not damaged by the initial injury. It has been suggested that the secondary degeneration results from diffusion of toxic chemicals produced by damaged neurons (McIntosh, 1993, *J. Neurotrauma* 10:215; Lynch and Dawson, 1994, Curr. Opin. Neurol. 7:510; Smith *et al.*, 1995, New Horiz. 3:562; Faden, 1996, Pharmacol. Toxicol., 78:12; Faden, 1996, *JAMA*, 276:569).

Popovitch *et al.* has shown that CNS trauma such as spinal injury triggers a systemic response to self epitopes such as myelin basic proteins (MBP) (Popovitch *et al.*, 1996, J. Neurosci. Res., 45:349). Activated T-cells that recognize a self antigen as well as activated T-cells that recognize a non-self antigen have been shown to enter the CNS parenchyma. Only T-cells capable of recognizing a CNS antigen appear to persist in the nervous tissue (Hickey *et al.*, 1991, *J. Neurosci. Res.* 28, 254-60). Although activated T-cells that recognize a self antigen (antiself T-cells) apparently persist in nervous tissue, use of activated T-cells that recognize a non-self antigen (non-self T-cells) for administration to an individual have advantages such as no risk of induction of autoimmune disease. Further, use of non-self activated T-cells eliminates the necessity of activating autologous or syngeneric T-cells; therefore, non-self T-cells may be activated and stored for use in any individual.

T-cells reactive to antigens of CNS white matter, such as myelin basic protein (MBP), can induce the paralytic disease experimental autoimmune encephalomyelitis (EAE) within several days of their inoculation into naive recipient rats (Ben Nun *et al.*, 1981, *Eur. J. Immunol.* 11, 195-9). Studies have suggested a role for anti-MBP T-cells in the human disease multiple sclerosis (Ota *et al.*, 1990, *Nature* 346, 183-7; Martin, 1997, *J. Neural. Transm. Suppl.* 49, 53-67; Sun, 1993, Acta Neurol. Scand. Suppl. 142:1-56). Despite their pathogenic potential, anti-MBP T cell clones are present in the immune systems of healthy subjects (Burns *et al.*, 1983, *Cell. Immunol.* 81, 435-40; Schluesener and

Wekerle, 1985, *J. Immunol.* 135, 3128-33). However, little is known about the possible physiological functions of antiself T-cells.

Citation or identification of any reference shall not be construed as an admission that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention is directed to methods and compositions for the treatment or diagnosis of injury of the central nervous system (CNS). The present invention provides methods for delivering a therapeutic or detectable substance to a site of injury or disease of the CNS, comprising administering an effective amount of activated T-cells that contain or express a therapeutic or detectable substance to a mammal wherein the amount is effective to detect, diagnose, or monitor a site of injury or disease in the CNS or is effective to ameliorate the effects of an injury or disease of the CNS. The activated T-cells used for delivery of a substance preferably do not recognize a nervous system specific antigen (NS-specific antigen); more preferably the activated T-cells recognize a non-self antigen. "Activated T-cell" as used herein includes (i) T-cells that have been activated by (a) exposure to a cognate antigen or derivative thereof or (b) exposure to an appropriate mitogen such as a lectin (e.g. concanavalin A (Con A) or phytohemagglutinin (PHA)), and (ii) progeny of such activated T-cells. As used herein, a cognate antigen is an antigen that is specifically recognized by the T-cell antigen receptor of a T-cell that has been previously exposed to the antigen. As used herein, a derivative of an antigen is a fragment or amino acid variant (*e.g.*, an insertion, substitution and/or deletion derivative) of the corresponding full-length antigen so long as the fragment or amino acid variant is capable of displaying one or more functional activities of the corresponding full-length antigen. Such functional activities include but are not limited to antigenicity

[ability to bind (or compete with the antigen for binding) to an anti-antigen-specific antibody], immunogenicity (ability to generate antibody which binds to the antigen), and ability to interact with T-cells resulting in activation comparable to that obtained using the corresponding full-length antigen.

The present invention also provides pharmaceutical compositions comprising a therapeutically effective amount of non-recombinant, NS-specific antiself T-cells and methods of use of such compositions for prevention or inhibition of CNS nerve degeneration in which the amount is effective to ameliorate the effects of an injury or disease of the CNS. "NS-specific antiself T-cell" as used herein refers to an activated T-cell which recognizes a self antigen present in a greater concentration in the nervous system (NS) compared to other organs or the circulation or an antigen that shares an antigenic determinant with an antigen present in a greater concentration in the NS compared to other organs or the circulation.

The present invention also provides pharmaceutical compositions and methods of use comprising a therapeutically effective amount of NS-specific antigens (or derivatives thereof) for prevention or inhibition of CNS degeneration in which the amount is effective to activate T-cells *in vivo* or *in vitro* wherein the activated T-cells ameliorate the effects of an injury or disease of the CNS. "NS-specific antigen" as used herein refers to an antigen present in the NS or an antigen that shares an antigenic determinant with an antigen present in a greater concentration in the NS compared to other organs or the circulation.

In the practice of the invention, therapy for amelioration of effects of injury or disease comprising administration of activated T-cells may optionally be in combination with NS-specific antiself T-cells or a NS-specific antigen (or derivative thereof) or NS-specific antiself T-cells and a NS-specific antigen.

4. BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 presents low-power epifluorescence micrographs of optic nerve after a controlled crush injury in rats treated with T-cell clones primed with MBP. See text, Section 6, for experimental details.

Fig. 2 presents high-power micrographs of the site of optic nerve injury shown in Fig. 1, showing the large concentration of injected cells localized in the site of injury.

Fig. 3 presents a serial section through non-injured optic nerve.

Fig. 4 is a graphical representation of the number of T-cells at the site of injury of two different T-cell clones primed with antigen MBP or ovalbumin (OVA) (T_{MBP} or T_{OVA}, respectively) after injury, at various time intervals following injury. T_{MBP} and T_{OVA} cells were injected into animals at the time of optic nerve crush, ipsolateral and contralateral nerves were then removed and prepared for microscopy at days 3, 7, 14 and 21. The figure shows that T-cells accumulated at the site of injury, independent of the antigen with which they were primed (each result is an average of 5 different experiments; the bar shows the standard deviation). See text, Section 6, for experimental details.

Fig. 5 shows accumulation of T-cells primed with MBP or OVA (T_{MBP} and T_{OVA}, respectively) measured immunochemically using antibodies to T-cell receptors. A comparison of the number of accumulated cells in injured optic nerve (ON) and in non-injured optic nerve is illustrated. See text, Section 6, for experimental details.

Fig. 6 shows accumulation of T-cells in injured and non-injured optic nerve after various treatment protocols. T-cells specific to MBP (T_{MBP}) were injected either immediately after nerve injury (T_{MBP} Cell Injection = 0) or 14 days after injury (T_{MBP} Cell Injection = 14). Their accumulation at the optic nerve was analyzed either 7 days (nerve excision - day 7) or 21 days (nerve excision = day 21) after injury.

Fig. 7 shows T-cell accumulation in injured optic nerve 1 week after injury. See text, Section 6, for experimental details. Anti-MBP or anti-OVA or anti-hsp60 T cell lines were raised, maintained, and activated by incubation with MBP from the spinal cords of guinea pigs, or with OVA (Sigma), or with the 51-70 peptide of MBP, respectively, in the presence of irradiated (2000 rad) syngeneic thymus cells. See text, Section 7, for experimental details. Activated T-cells ($1x10^7$ cells) of the anti-MBP or anti-OVA lines or PBS were injected intraperitoneally into adult Lewis rats immediately after unilateral crush injury of the optic nerve. Seven days after injury both optic nerves were removed, cryosectioned, and analyzed immunohistochemically for the presence of labeled T-cells. Bars shows the mean total numbers of T-cells counted in 2 or 3 sections of each nerve. Each group contained 3 or 4 rats.

Fig. 8 illustrates inhibition of secondary degeneration after partial optic nerve crush injury in adult rats. See text, Section 7, for experimental details. Surviving optic nerve fibers were monitored by retrograde labeling of retinal ganglion cells immediately after injury and 2 weeks later. Five days after dye application retinas were excised, and labeled retinal ganglion cells (RGCs) were counted under the fluorescence microscope. Counting was performed in 5 randomly selected fields in each retina (all located at approximately the same distance from the optic disc). The number of RGCs in each group of injured nerves in rats injected with PBS only or injected with anti-MBP, anti-OVA, or anti-hsp60 T-cells was expressed as percentages of the total numbers of spared neurons following the primary injury (42% of the axons remained viable after the primary injury).

Fig. 9 presents photomicrographs showing retrograde labeled retinas of injured optic nerves of rats injected with (A) PBS, (B) anti-hsp60 T-cells, or (C) anti-MBP T-cells. See text. Section 7, for experimental details.

Fig. 10 shows number of surviving RGCs of injured optic nerves of rats injected with anti-MBP T-cells (T_{MBP}), T-cells

generated against a peptide comprising amino acids 51-70 of MBP (T_p 51-70), or PBS. See text, Section 7, for experimental details.

Fig. 11 presents clinical course of rats injected with anti-MBP T-cells. Results were evaluated according to the neurological paralysis scale (EAE score). Rats were injected i.p. with $1x10^7$ activated anti-MBP T-cells either immediately after optic nerve crush injury (-■-) or without optic nerve crush injury (--o--). Each group contained 5 to 9 rats. Data points represents means ± SEM. See text, Section 7, for experimental details.

Fig. 12 shows the survival of neurons in uninjured nerves in rats injected i.p. with $1x10^7$ activated anti-MBP T-cells or PBS. See text. Section 7, for experimental details.

Fig. 13 illustrates inhibition of secondary degeneration after optic nerve crush injury in adult rats. See text, Section 8, for experimental details. Rats were injected intradermally into footpads with a 21-mer peptide based on amino acid residues 35-55 (MOG p35-55) of myelin/oligodendrocyte glycoprotein (chemically synthesized at the Weizmann Institute, Israel) (50 μg/animal) or PBS ten days prior to optic nerve crush injury or MOG p35-55 in the absence of crush injury. MOG p35-55 was administered with Incomplete Freund's Adjuvant. Surviving optic nerve fiber were monitored by retrograde labeling of retinal ganglion cells (RGCs). The number of RGCs in rats injected with PBS or MOG p35-55 was expressed as a percentage of the total number of neurons in rats injected with MOG p35-55 in the absence of crush injury.

Fig. 14 illustrates inhibition of secondary degeneration after optic nerve crush injury in adult rats. See text, Section 9, for experimental details. MBP (Sigma, Israel) (1 mg in 0.5 ml saline) was administered to adult rats by gavage using a blunt needle MBP was administered 5 times, i.e., every third day beginning 2 weeks prior to optic nerve crush injury. Surviving optic nerve fibers were monitored by retrograde labeling of retinal ganglion cells (RGCs). The

number of RGCs in treated rats was expressed as a percentage of the total number of neurons in untreated rats following the injury.

5. DETAILED DESCRIPTION OF THE INVENTION

In the practice of the invention, compositions comprising activated T-cells are used for delivery of (a) a diagnostic substance or (b) a therapeutic substance to a site of injury or disease of the CNS in a mammal.

Generally, T-cells of the present invention are T-cells which recognize an antigen not normally present or present in small quantities in the circulation. Such antigens include but are not limited to NS-specific antigens, cryptic antigens or "non-self" antigens (*i.e.*, antigens not normally present in an individual). Non-self antigens may be, without limitation, viral, bacterial, etc., including tissue-specific antigens from a different species or individual.

In an embodiment, T-cells are activated *in vitro* by exposure to an antigen and administered to a mammal.

The present invention provides methods for delivering a therapeutic or detectable substance to a site of injury or disease of the CNS, comprising administering an effective amount of activated T-cells that contain or express a therapeutic or detectable substance to a mammal wherein the amount is effective to detect, diagnose, or monitor a site of injury or disease in the CNS or is effective to ameliorate the effects of an injury or disease of the CNS.

Pharmaceutical compositions comprising NS-specific antiself T-cells and methods of use of such compositions for prevention or inhibition of CNS degeneration are provided. In a preferred embodiment, the NS-specific antiself T-cells are non-recombinant.

Pharmaceutical compositions and method of use thereof comprising a NS-specific antigen (or derivative thereof) are used for preventing or inhibiting degeneration of nerves within the CNS.

The present invention provides methods for (a) delivery of substances to a site of CNS injury or disease comprising administration of activated T-cells and (b) amelioration of degeneration comprising administration of (i) NS-specific antiself T-cells or (ii) an NS-specific antigen or derivative thereof or both (i) and (ii). In the practice of the invention, substance-delivering activated T-cells may optionally be administered in combination with (a) NS-specific antiself T-cells or (b) an NS-specific antigen (or derivative thereof) or both (a) and (b).

If desired, the methods of the present invention may optionally be combined concurrently with one or more of the following: (a) administration into the CNS of mononuclear phagocytes (preferably cultured monocytes) that have been stimulated to enhance their capacity to promote axonal regeneration; (b) administration into the CNS of a neurotrophic factor such as acidic fibroblast growth factor; and (c) administration of an anti-inflammatory therapeutic substance (*i.e.*, an anti-inflammatory steroid, such as dexamethasone or methylprednisolone, or a non-steroidal anti-inflammatory agent or drug, such as aspirin, indomethacin, ibuprofen, fenoprofen, ketoprofen or haproxen, or an anti-inflammatory peptide, such as Thr-Lys-Pro (TKP)).

5.1 DELIVERY OF SUBSTANCES

Described herein are activated T-cells capable of delivering a diagnostic or therapeutic substance to a site of injury or disease in the CNS. In a preferred embodiment the activated T-cells do not recognize a NS-specific antigen; more preferably, the activated T-cells recognize a non-self antigen. In one embodiment, such activated T-cells may be used as part of a diagnostic technique for the detection of a site of damage in the CNS caused by injury or disease. In another embodiment, the activated T-cells may be used as part of a therapeutic regimen for ameliorating the effects of injury or disease of the CNS by promoting axonal regeneration or inhibiting or preventing CNS degeneration.

5.1.1 DIAGNOSTIC AND THERAPEUTIC COMPOSITIONS

Activated T-cells of the present invention can be used for the delivery of various therapeutic and detectable substances to a site of injury or disease within the CNS. In a preferred embodiment, the activated T-cells of the present invention are activated by exposure to an antigen that is not NS-specific, more preferably by exposure to a non-self antigen. The detectable substances may be used for detecting, diagnosing or monitoring a site of injury or disease of the CNS.

In an embodiment, the T-cells are allogeneic T-cells, *e.g.* a pooled T-cell preparation obtained from a blood bank. The use of allogeneic T-cells is applicable for various treatments comprising limited administrations, including but not limited to, delivery of T-cells to a site of CNS injury for diagnostic purposes; for an acute single administration or one-dose therapy, etc. In another embodiment, the T-cells are syngeneic T-cells, preferably autologous T-cells (*i.e.*, from the same individual).

T-cells can be isolated and purified according to methods known in the art (Mor and Cohen, 1995, J. Immunol. 155:3693-3699). For an illustrative example, see Section 6.1.

For use in the diagnostic methods of the invention, T-cells which preferentially localize to a site of injury or disease in the CNS can be detectably labeled.

The T-cells can be detectably labeled with a contrast agent including, without limitation, metals such as gold particles, gadolinium complexes, etc. Alternatively, the T-cells can be labeled detectably with a radioisotope, including but not limited to: ^{125}Iodine, ^{131}Iodine, ^{99m}Technecium. The T-cells can also be detectably labeled using a fluorescence emitting metal such as ^{152}Eu, or others of the lanthanide series.

Methods for detectably labeling T-cells may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory

Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York) and Current Protocols in Immunology (Current Protocols in Immunology, 1997, Eds., Coligan et al., John Wiley & Sons, Inc., NIH) which are incorporated herein by reference in their entirety. Labeling of T-cells with metal particles may be achieved by incubating cells in a suspension comprising the metal particles wherein the T-cells spontaneously internalize such particles into the cell's cytosol. Such substances may also be introduced into the cells by a variety of electroporetic techniques (Current Protocols in Immunology, 1997, Eds. Coligan et al., John Wiley & Sons, Inc., NIH). Fluorescence emitting metals or radioactive metals can be attached to the T-cells using such metal chelating agents as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA). Labeling of T-cells with a radioisotope can be achieved by incubating cells with a radioactive metabolic precursor.

Presence of labeled, activated T-cells can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include but are not limited to: computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), sonography, radiation responsive surgical instrument (Thurston *et al.*, U.S. Patent 5,441,050), and fluorescence responsive scanning instrument.

After labeling, the T-cells of the present invention are activated. The T-cells may be activated by exposure of the cells to one or more of a variety of natural and synthetic antigens and epitopes, including but not limited to, lipopolysaccharide (LPS), myelin basic protein (MBP), myelin/oligodendrocyte glycoprotein (MOG), myelin proteolipid protein (PLP), myelin associated protein (MAG), S-100, ß-amyloid, Thy-1, neurotransmitter receptors. Preferably, T-

cells are activated by an antigen that is not specific to the NS, more preferably by a non-self antigen.

During *ex vivo* activation of the T-cells, the T-cells may be activated by culturing them in medium to which at least one suitable growth promoting factor has been added. Growth promoting factors suitable for this purpose include, without limitation, cytokines, for instance tumor necrosis factor α (TNF-α), interleukin 2 (IL-2), and interleukin 4 (IL-4).

In an embodiment, the activated T-cells endogenously produced a substance that ameliorates the effects of injury or disease in the CNS.

In another embodiment, the activated T-cells endogenously produce a substance that stimulates other cells, including, but not limited to, transforming growth factor-ß (TGF-ß), nerve growth factor (NGF), neurotrophic factor 3 (NT-3), neurotrophic factor 4/5 (NT-4/5), brain-derived neurotrophic factor (BDNF), interferon-δ (IFN-δ), interleukin-6 (IL-6), wherein the other cells, directly or indirectly, ameliorate the effects of injury or disease.

In another embodiment, the T-cells may be genetically engineered *in vitro* to insert therein a nucleotide sequence as described in Kramer *et al.*, 1995, Nature Medicine, 1(11):1162-1166. The nucleotide sequence is under the control of necessary elements for transcription and translation such that a biologically active protein encoded by the nucleotide sequence can be either expressed continuously or induced to expression as a result of exposure of the T-cells to a microenvironment of a kind present at the site of injury.

Due to the inherent degeneracy of the genetic code, other nucleotide sequences that encode substantially the same or a functionally equivalent amino acid sequence of a protein, are within the scope of the invention. Preferably, the expression product of said nucleotide sequence is a secretory protein.

The recombinant T-cells which contain a coding sequence and which express a biologically active gene product may be identified by at least four general approaches: (a) DNA-DNA or DNA-RNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of transcription as measured by the expression of mRNA transcripts in the cell; and (d) detection of the product encoded by the nucleotide sequence as measured by immunoassay or by its biological activity.

In the first approach, the presence of the coding sequence inserted in the expression vector can be detected by DNA-DNA or DNA-RNA hybridization using probes comprising nucleotide sequences that are homologous to the coding sequence or portions or derivatives thereof.

In the second approach, the recombinant expression system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the coding sequence is inserted within a marker gene sequence of a vector, recombinant cells containing the coding sequence can be identified by the absence of the marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence under the control of the same or different promoter used to control the expression of the coding sequence. Expression of the marker in response to induction or selection indicates expression of the coding sequence.

In the third approach, transcriptional activity of a nucleotide sequence can be assessed by hybridization assays. For example, RNA can be isolated and analyzed by Northern blot using a probe having sequence homology to a coding sequence or transcribed noncoding sequence or particular portions thereof. Alternatively, total nucleic acid of the host cell may be extracted and quantitatively assayed for hybridization to such probes.

In the fourth approach, the levels of a protein product can be assessed immunologically, for example by Western blots, immunoassays such as radioimmuno-precipitation, enzyme-linked immunoassays and the like.

The T-cells may be stably transfected with said nucleotide sequences or may be transiently transfected. Transient transfection may be applicable for acute one-dose therapeutic regimens.

Such nucleotide sequences may encode various substances including, without limitation, therapeutic substances; enzymes which catalyze a therapeutic substance; a regulatory product which stimulates expression of a therapeutic substance in the T-cells, etc. Examples include: nucleotide sequences encoding neurotrophic factors such as NGF; nucleotide sequences encoding enzymes which play a role in CNS nerve regeneration such as the enzyme transglutaminase; nucleotide sequences encoding enzymes which catalyze the production of a neurotransmitter, *e.g.* enzymes involved in the catalysis of acetylcholine or dopamine, etc. As a result, T-cells which localize at the site of CNS injury or disease produce and secrete the needed substances at the site.

As will be evident to those skilled in the art, the T-cells can be preserved, *e.g.* by cryopreservation, either before or after culture.

Cryopreservation agents which can be used include but are not limited to dimethyl sulfoxide (DMSO) (Lovelock and Bishop, 1959, Nature 183:1394-1395; Ashwood-Smith, 1961, Nature 190:1204-1205), glycerol, polyvinylpyrrolidone (Rinfret, 1960, Ann. N.Y. Acad. Sci. 85:576), polyethylene glycol (Sloviter and Ravdin, 1962, Nature 196:548), albumin, dextran, sucrose, ethylene glycol, i-erythritol, D-ribitol, D-mannitol (Rowe et al., 1962, Fed. Proc. 21:157), D-sorbitol, i-inositol, D-lactose, choline chloride (Bender et al., 1960, J. Appl. Physiol. 15:520), amino acids (Phan The Tran and Bender, 1960, Exp. Cell Res. 20:651), methanol, acetamide, glycerol monoacetate (Lovelock, 1954, Biochem. J.

56:265), inorganic salts (Phan The Tran and Bender, 1960, Proc. Soc. Exp. Biol. Med. 104:388; Phan The Tran and Bender, 1961, in Radiobiology, Proceedings of the Third Australian Conference on Radiobiology, Ilbery, P.L.T., ed., Butterworth, London, p. 59), and DMSO combined with hydroxyethel starch and human serum albumin (Zaroulis and Leiderman, 1980, Cryobiology 17:311-317).

A controlled cooling rate is critical. Different cryoprotective agents (Rapatz et al., 1968, Cryobiology 5(1):18-25) and different cell types have different optimal cooling rates. See, e.g., Rowe and Rinfret, 1962, Blood Transfusion 7(1):17-32; and Mazur, 1970, Science 168:939-949 for effects of cooling velocity on survival of cells and on their transplantation potential. The heat of fusion phase where water turns to ice should be minimal. The cooling procedure can be carried out by use of, e.g., a programmable freezing device or a methanol bath procedure. Programmable freezing apparatuses allow determination of optimal cooling rates and facilitate standard reproducible cooling. Programmable controlled-rate freezers such as Cryomed or Planar permit tuning of the freezing regimen to the desired cooling rate curve.

After thorough freezing, cells can be rapidly transferred to a long-term cryogenic storage vessel. In one embodiment, samples can be cryogenically stored in mechanical freezers, such as freezers that maintain a temperature of about -80°C or about -20°C. In a preferred embodiment, samples can be cryogenically stored in liquid nitrogen (-196°C) or its vapor. Such storage is greatly facilitated by the availability of highly efficient liquid nitrogen refrigerators, which resemble large Thermos containers with an extremely low vacuum and internal super insulation, such that heat leakage and nitrogen losses are kept to an absolute minimum.

Considerations and procedures for the manipulation, cryopreservation, and long term storage of T-cells can be

found, for example, in the following references, incorporated by reference herein: Gorin, 1986, Clinics in Haematology 15(1):19-48; Bone-Marrow Conservation, Culture and Transplantation, Proceedings of a Panel, Moscow, July 22-26, 1968, International Atomic Energy Agency, Vienna, pp. 107-186.

Other methods of cryopreservation of viable cells, or modifications thereof, are available and envisioned for use, *e.g.*, cold metal-mirror techniques. See Livesey and Linner, 1987, Nature 327:255; Linner et al., 1986, J. Histochem. Cytochem. 34(9):1123-1135; see also U.S. Patent No. 4,199,022 by Senken et al., U.S. Patent No. 3,753,357 by Schwartz, U.S. Patent No. 4,559,298 by Fahy.

Frozen cells are preferably thawed quickly (*e.g.*, in a water bath maintained at 37-41°C) and chilled immediately upon thawing. It may be desirable to treat the cells in order to prevent cellular clumping upon thawing. To prevent clumping, various procedures can be used, including but not limited to the addition before or after freezing of DNAse (Spitzer et al., 1980, Cancer 45:3075-3085), low molecular weight dextran and citrate, hydroxyethyl starch (Stiff et al., 1983, Cryobiology 20:17-24), or acid citrate dextrose (Zaroulis and Leiderman, 1980, Cryobiology 17:311-317), etc.

The cryoprotective agent, if toxic in humans, should be removed prior to therapeutic use of the thawed T-cells. One way in which to remove the cryoprotective agent is by dilution to an insignificant concentration.

Once frozen T-cells have been thawed and recovered, they are used to promote axonal regeneration as described herein with respect to non-frozen T-cells.

5.1.2 USES

The compositions and methods of the present invention comprising activated, substance-delivering T-cells are useful for treating or detecting a site of damage in the CNS caused by injury or disease.

Methods for detecting a site of injury or disease of the CNS in a mammal comprise: (a) administering to a mammal an effective amount of labeled activated T-cells; and (b) detecting in the mammal the labeled activated T-cells that have accumulated at said site of injury or disease, in which step (b) is performed after an interval sufficient to permit said labeled activated T-cells administered to step (a) to accumulate at said site of injury or disease.

For use in the therapeutic methods of the invention, activated T-cells can be used to deliver substances for ameliorating the effects of injury or disease by, for example, promoting axonal regeneration or inhibiting or preventing degeneration of the CNS. Such substances include, without limitation, growth factors which promote nerve regeneration such as nerve growth factor (NGF); substances lacking at the site of injury, *e.g.* neurotransmitters such as acetylcholine, dopamine; anti-inflammatory substances, etc. Further, activated T-cells may endogenously produce a substance that has a therapeutic effect on the CNS injury, including, without limitation, interleukins and growth factors.

In a preferred embodiment, the activated T-cells do not recognize a NS-specific antigen; more preferably, the labeled activated T-cells recognize a non-self antigen.

The injury or disease may be situated in any portion of the CNS, including the brain, spinal cord, or optic nerve. One example of such injury or disease is trauma, including blunt trauma, penetrating trauma, and trauma sustained during a neurosurgical operation or other procedure. Another example of such injury or disease is stroke, including hemorrhagic stroke and ischemic stroke. Other examples of disease are Alzheimer's disease, multiple sclerosis, Huntington's disease, ALS, and Parkinson's disease. Yet another example of such injury or disease is optic nerve injury accompanying optic neuropathy or glaucoma. Still further examples of CNS injury or disease will be evident to those skilled in the art from this description and are

encompassed by the present invention. The compositions and methods of the present invention are useful for treating CNS injury or disease that results in axonal damage whether or not the subject also suffers from another disease of the central or peripheral nervous system, such as neurological disease of genetic, metabolic, toxic, nutritional, infective or autoimmune origin.

5.2 AMELIORATION OF CNS DAMAGE

5.2.1 THERAPEUTIC COMPOSITIONS AND USES

The invention also provides methods of preventing or inhibiting CNS degeneration by administering a composition comprising an effective amount of NS-specific antiself T-cells. In a preferred embodiment the NS-specific antiself T-cells are non-recombinant cells.

The invention also provides methods of preventing or inhibiting CNS degeneration by administering a composition comprising an effective amount of a NS-specific antigen.

The activated, substance-delivering T-cells described, *supra*, in Section 5.1 may be used alone or in combination with NS-specific antiself T-cells or a NS-specific antigen or NS-specific antiself T-cells and a NS-specific antigen for ameliorating the effects of injury or disease, *e.g.*, for promoting axonal regeneration and preventing or inhibiting CNS degeneration.

5.2.1.1 NS-SPECIFIC ANTISELF T-CELLS

NS-specific antiself T-cells (ATCs) can be used for ameliorating the effects of injury or disease of the CNS that result in CNS degeneration. In a preferred embodiment the NS-specific antiself T-cells are isolated.

Circulating T-cells of a subject which recognize myelin basic protein or another NS antigen such as the amyloid precursor protein are isolated and expanded using known procedures. In order to obtain NS-specific antiself T-cells, T-cells are isolated and the NS-specific ATCs are then expanded by known procedures (Burns *et al.*, *Cell Immunol.*

81:435 (1983); Pette *et al.*, *Proc. Natl. Acad.* Sci. USA 87:7968 (1990); Mortin *et al.*, *J. Immunol.* 145:540 (1990); Schluesener *et al.*, *J. Immunol.* 135:3128 (1985); Suruhan-Dires Keneli *et al.*, *Euro. J. Immunol.* 23:530 (1993) which are incorporated herein by reference in their entirety.

Following their proliferation *in vitro*, the T-cells are administered to a mammalian subject. In a preferred embodiment, the T-cells are administered to a human subject. T-cell expansion is preferably performed using peptides corresponding to sequences in a non-pathogenic, NS-specific, self protein.

A subject can initially be immunized with a NS-specific antigen using a non-pathogenic peptide of the self protein. A T-cell preparation can be prepared from the blood of such immunized subjects, preferably from T-cells selected for their specificity towards the NS-specific antigen. The selected T-cells can then be stimulated to produce a T-cell line specific to the self-antigen (Ben-Nun *et al.*, *J. Immunol.* 129:303 (1982)).

The NS-specific antigen may be a purified antigen, a crude NS preparation, or a peptide derived from a NS-antigen, as will be described below.

NS-specific ATCs, obtained as described above, can be used immediately or may be preserved for later use, *e.g.* by cryopreservation as described in Section 5.1, *supra*. NS-specific ATCs may also be obtained using previously cryopreserved T-cells, *i.e.*, after thawing the cells, the T-cells may be incubated with NS-specific antigen, optimally together with thymocytes, to obtain a preparation of NS-specific ATCs.

5.2.1.2 NS-SPECIFIC ANTIGENS

Pharmaceutical compositions comprising a NS-specific antigen are used for ameliorating the effects of injury or disease that result in CNS degeneration. Additionally, NS-specific antigens may be used for *in vivo* or *in vitro* activation of antiself T-cells. In an embodiment, the NS-

specific antigen is an isolated antigen. In an embodiment, methods of ameliorating the effects of CNS injury or disease comprise administering NS-specific antigen to a mammal wherein the NS-specific Ag activates T-cells *in vivo* to produce a population of T-cells that accumulate at a site of injury or disease of the CNS.

The NS-specific antigen may be an antigen obtained from NS tissue, preferably from tissue at a site of CNS injury or disease. The NS-specific antigen may be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of antigens. The functional properties may be evaluated using any suitable assay.

In a preferred embodiment, peptides derived from NS-specific, self antigens activate T-cells, but do not induce an autoimmune disease. An example of such a antigen fragment is a peptide comprising amino acids 51-70 of myelin basic protein. SEQ ID NO:1 (Kamholz *et al.*, 1986, Proc. Natl. Acad. Sci. USA 83:4962-4966, GenBank accession number M13577; Roth *et al.*, 1987, J. Neurosci. Res. 17(4):321-328, GenBank accession number M30516).

In addition, a NS-specific antigen may be a crude NS-tissue preparation, *e.g.*, derived from tissue obtained at the site of CNS injury. Such a preparation may include cells, both living or dead cells, membrane fractions of such cells or tissue, etc.

A NS-specific antigen may be obtained by a NS biopsy from a mammal including, but not limited to, from a site of CNS injury; from cadavers; from cell lines grown in culture. Additionally, a NS-specific antigen may be a protein obtained by genetic engineering, chemically synthesized, etc.

In addition to purified antigen, the invention also relates to derivatives (*e.g.*, fragments) or analogs of NS-specific antigens which are functionally active, *i.e.*, they are capable of displaying one or more known functional

activities associated with a full-length NS-specific antigen. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with a CNS-antigen for binding) to an anti-NS-specific antibody], immunogenicity (ability to generate antibody which binds to a NS-specific protein), and ability to interact with T-cells, resulting in activation comparable to that obtained using the corresponding full-length antigen.

In a specific embodiment of the invention, proteins consisting of or comprising a fragment of a NS-specific antigen consisting of at least 10 (contiguous) amino acids of the CNS-specific antigen is provided. In other embodiments, the fragment consists of at least 20 contiguous amino acids or 50 contiguous amino acids of the NS-specific antigen. Derivatives or analogs of a NS-specific antigen include but are not limited to those molecules comprising regions that are substantially homologous to the full-length antigen or fragments thereof (*e.g.*, in various embodiments, at least 60% or 70% or 80% or 90% or 95% identity over an amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art) or whose encoding nucleic acid is capable of hybridizing to a coding nucleotide sequence of the full-length NS-specific antigen, under stringent, moderately stringent, or nonstringent conditions.

The NS-specific antigen derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, a cloned gene sequence can be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*.

Additionally, the coding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical mutagenesis, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem 253:6551), etc.

Manipulations may also be made at the protein level. Included within the scope of the invention are fragments or other derivatives or analogs which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In addition, analogs and derivatives of a NS-specific antigen can be chemically synthesized. For example, a peptide corresponding to a portion of an antigen which comprises the desired domain or which mediates the desired activity can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the amino acid sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-

butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The functional activity of NS-specific antigens and derivatives thereof can be assayed by various methods known in the art, including, but not limited to T-cell proliferation assays (Mor and Cohen, 1995, J. Immunol. 155:3693-3699).

A NS-specific antigen or derivative thereof may be kept in solution or may be provided in a dry form, *e.g.* as a powder or lyophilizate, to be mixed with appropriate solution prior to use.

5.2.2 USES

The compositions described in Section 5.2 may be used to prevent or inhibit secondary degeneration which may otherwise follow primary CNS injury, *e.g.* a cut or a crush in a CNS tissue. In addition, such compositions may be used to ameliorate the effects of disease that results in degenerative processes, *e.g.* degeneration occurring in either grey or white matter (or both) as a result of various diseases or disorders including, without limitation: senile dementias, Alzheimer's disease, Parkinson's Disease, glaucoma, multiple sclerosis, Huntington's disease, ALS, prion diseases such as Creutzfeldt-Jakob disease, etc.

5.3 FORMULATIONS AND ADMINISTRATION

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. The carriers in the pharmaceutical composition may comprise a binder, such as microcrystalline cellulose, polyvinylpyrrolidone (polyvidone or povidone), gum tragacanth, gelatine, starch, lactose or lactose monohydrate; a disintegrating agent, such as alginic acid, maize starch and the like; a lubricant or surfactant, such as magnesium stearate, or sodium lauryl sulphate; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; and/or a flavoring agent, such as peppermint, methyl salicylate, or orange flavoring.

Methods of administration include, but are not limited to, parenteral *e.g.* intravenous, intraperitoneal, intramuscular, subcutaneous, and mucosal *e.g.*, oral, nasal, buccal, vaginal, rectal, intraocular) routes. Administration can be systemic or local.

For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents

(*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well-known in the art.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

For administration by inhalation, the compositions for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

In a preferred embodiment, compositions comprising substance-delivering activated T-cells or NS-specific antiself T-cells are formulated in accordance with routine

procedures as pharmaceutical compositions adapted for intravenous or intraperitoneal administration to human beings.

Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water or saline for injection can be provided so that the ingredients may be mixed prior to administration.

In an embodiment, pharmaceutical compositions comprising NS-specific antigen are administered with an adjuvant, such as Incomplete Freund's Adjuvant.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention.

In a preferred embodiment, the pharmaceutical compositions of the invention are administered to a mammal shortly after injury or detection of a degenerative lesion in the CNS. The therapeutic methods of the invention may comprise administration of activated T-cells or NS-specific ATCs or a NS-specific antigen or any combination thereof.

In an embodiment, the NS-specific ATCs or a NS-specific antigen of the invention are administered in combination with a therapeutic composition which promotes regeneration of axons within the CNS; the latter therapeutic composition may comprise for example, the activated, substance-delivering T-cells of the present invention wherein the said substance promotes nerve regeneration. Alternatively, the therapeutic composition may comprise mononuclear phagocytes as described in PCT Publication No. WO 97/09985, which is incorporated

herein by reference in its entirety. Briefly, mononuclear phagocytes which are cultured *ex vivo* together with a stimulatory tissue, such as dermis or a nerve segment, are administered into the central nervous system of a mammal at or near the site of injury or disease-inflicted lesion. In an embodiment, the mononuclear phagocytes are allogeneic. In a preferred embodiment, the mononuclear phagocytes are autologous.

In an embodiment, mononuclear phagocyte cells according PCT Publication No. WO 97/09985 and U.S. patent application Serial No. 09/041,280, filed March 11, 1998, are injected into the site of injury or lesion within the CNS, either concurrently, prior to, or following parenteral administration of NS-specific ATCs or NS-specific antigen.

In an embodiment, administration of substance-delivering activated T-cells, or NS-specific ATCs, a NS-specific antigen, may be administered as a single dose or may be repeated, preferably at 2 week intervals and then successively longer intervals once a month, once a quarter, once every six months, etc. The course of treatment may last several months, several years or occasionally also through the life-time of the individual, depending on the condition or disease which is being treated. In the case of a CNS injury, the treatment may range between several days to months or even years, until the condition has stabilized and there is no or only a limited risk of development of secondary degeneration. In chronic human diseases or conditions such as Alzheimer's disease or Parkinson's disease, the therapeutic treatment in accordance with the invention may be for life.

As will be evident to those of skill in the art, the therapeutic effect depends at times on the condition or disease to be treated, on the individual's age and health condition, on other physical parameters (*e.g.* gender, weight, etc.) of the individual, as well as on various other factors, *e.g.* whether the individual is taking other drugs, etc.

The optimal dose of the therapeutic compositions comprising activated T-cells or NS-specific antiself T-cells of the invention is proportional to the number of nerve fibers affected by CNS injury or disease at the site being treated. In a preferred embodiment, the dose ranges from about 5 x 10^6 to about 10^7 for treating a lesion affecting about 10^5 nerve fibers, such as a complete transection of a rat optic nerve, and ranges from about 10^7 to about 10^8 for treating a lesion affecting about 10^5 nerve fibers, such as a complete transection of a human optic nerve. As will be evident to those of skill in the art, the dose of T-cells can be scaled up or down in proportion to the number of nerve fibers affected at the lesion or site of injury being treated.

6. EXAMPLE: ACCUMULATION OF ACTIVATED T-CELLS IN INJURED CNS

6.1 MATERIALS AND METHODS

6.1.1 ANIMALS

Female Lewis rats were obtained from Harlan Olac (Bicester, UK), matched for age (8-12 weeks) and housed four to a cage in a light and temperature-controlled room.

6.1.2 PROTEINS USED FOR T-CELL ACTIVATION

Myelin basic protein (MBP) was prepared from guinea pig spinal cord as previously described (Ben-Nun *et al., supra* (1982)). Chick ovalbumin (OVA) was purchased from Sigma (Israel). Heat-inactivated *Mycobacterium tuberculosis* H37RA (*M. tuberculosis*) and Incomplete Freund's adjuvant (IFA) were purchased from Difco Laboratories (Detroit, MI, USA).

6.1.3 MEDIA

The proliferation medium of the T-cells contained the following: Dulbecco's modified Eagle's medium (DMEM, Biological Industries, Israel) supplemented with 2mM L-glutamine (L-Glu, Sigma, USA), $5x10^{-5}$M 2-mercaptoethanol (2-

ME, Sigma), penicillin (100 IU/ml; Biological Industries), streptomycin (100 μg/ml; Biological Industries), sodium pyruvate (1 mM; Biological Industries), non-essential amino acids (1 ml/100 ml; Biological Industries) and autologous rat serum 1% (vol/vol) (Mor *et al.*, *Clin. Invest.*, 85:1594 (1990)). Propagation medium contained: DMEM, 2-ME, L-Glu, sodium pyruvate, non-essential amino acids and antibiotics in the same concentration as above and also 10% fetal calf serum (FCS), and 10% T-cell growth factor (TCGF) obtained from the supernatant of concanavalin A-stimulated spleen cells (Mor *et al.*, *supra*, 1990).

6.1.4 ANTIGENS

MBP from the spinal cords of guinea pigs was prepared as described (Hirshfeld, *et al.*, 1970, *FEBS Lett.* 7:317). OVA was purchased from Sigma (St. Louis, Missouri). The p51-70 of the rat 18.5kDa isoform of MBP (sequence: APKRGSGKDSHTRTTHYG) SEQ ID NO:2 and the p277 of the human hsp60 (sequence: VLGGGCALLRCPALDSLTPANED) SEQ ID NO:3 (Elias, *et al.*, 1991, *Proc. Natl. Acad. Sci. USA 88*, 3088-91) were synthesized using the 9-fluorenylmethoxycarbonyl technique with an automatic multiple peptide synthesizer (AMS 422, ABIMED, Langenfeld, Germany). The purity of the peptides was analyzed by HPLC and amino acid composition.

6.1.5 T CELL LINES

T-cell lines were generated from draining lymph node cells obtained from Lewis rats immunized with an antigen. The antigen was dissolved in PBS (1mg/ml) and emulsified with an equal volume of incomplete Freund's adjuvant (Difco Laboratories, Detroit, Michigan) supplemented with 4 mg/ml *Mycobacterium tuberculosis* (Difco). The emulsion (0.1 ml) was injected into hind foot pads of the rats. Ten days after the antigen was injected, the rats were killed and draining lymph nodes were surgically removed and dissociated. The cells were washed and activated with the antigen (10μg/ml) in proliferation medium containing Dulbecco's modified Eagle's

medium (DMEM) supplemented with L-glutamine (2mM), 2-mercaptoethanol ($5x10^{-5}$M), sodium pyruvate (1mM), penicillin (100 IU/ml), streptomycin (100 μg/ml), nonessential amino acids (1 ml/100 ml) and autologous rat serum 1% (volume/volume). After incubation for 72 h at 37°C, 90% relative humidity and 7% CO_2, the cells were transferred to propagation medium which additionally contained 10% fetal calf serum (FCS) (volume/volume) and 10% T cell growth factor derived from the supernatant of concanavalin A-stimulated spleen cells. Cells were grown in propagation medium for 4-10 days before being re-exposed to antigen (10μg/ml) in the presence of irradiated (2000 rad) thymus cells (10^7 cells/ml) in proliferation medium. The T-cell lines were expanded by repeated re-exposure and propagation.

6.1.6 LABELING OF T-CELLS

T-cells were washed and suspended in 10.7μm Hoechst 33342 Stain (Molecular Probes, USA) for 10 minutes at 37°C. The cells were washed twice with 50 ml volumes of PBS and then resuspended at 5 x 10^6 cells/ml on ice until injection.

6.1.7 CRUSH INJURY OF RAT OPTIC NERVE

Crush injuries were performed as previously described (Hirschberg *et al.*, 1994, J. Neuroimmunol. 50:9-16). Briefly, rats were deeply anesthetized by i.p. injection of xylazine (10 mg/kg; Rompun) and ketamine (50 mg/kg; Velalar). Under a binocular operating microscope, a lateral canthotomy was performed in the right eye and the conjunctiva was incised lateral to the cornea. After separation of the retractor bulbi muscles, the optic nerve was exposed intraorbitally by blunt dissection. A moderate crush injury was inflicted on the optic nerve, 2 mm from the eye, using a calibrated cross-action forceps (Duvdevani *et al.*, *Instructure Neurology and Neuroscience*, 2:31, 1990). The contralateral nerve was left undisturbed and was used as a control.

L16 ANSWER 5 OF 49 CAPLUS COPYRIGHT 2002 ACS
AN 2002:675821 CAPLUS
DN 137:222033
TI Compositions and methods for enhancing drug delivery across and into ocular tissues
IN Rothbard, Jonathan B.; Wender, Paul A.; McGrane, P. Leo; Sista, Lalitha Vs; Kirschberg, Thorsten A.
PA Cellgate, Inc., USA
SO PCT Int. Appl., 119 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002067917	A1	20020906	WO 2002-US5804	20020225
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	US 2002127198	A1	20020912	US 2001-792480	20010223
PRAI	US 2001-792480	A	20010223		
	US 1999-150510P	P	19990824		
	US 2000-648400	A2	20000824		

OS MARPAT 137:222033
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 6 OF 49 CAPLUS COPYRIGHT 2002 ACS
AN 2002:655010 CAPLUS
DN 137:195551
TI Recombinant .alpha. monomer of type IV collagen for inhibiting angiogenesis and treating neovascularization, tumor and metastasis
IN Hudson, Billy G.; Sarras, Michael P., Jr.
PA University of Kansas Medical Center, USA
SO U.S., 36 pp., Cont-in-part of U. S. Ser. No.183,548.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 5

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6440729	B1	20020827	US 1999-277665	19990326
	US 5691182	A	19971125	US 1995-497206	19950630
	US 5856184	A	19990105	US 1997-800965	19970218
	US 6384012	B1	20020507	US 1998-183548	19981030
	US 6432706	B1	20020813	US 2000-589927	20000607
	US 6498140	B1	20021224	US 2000-589987	20000607
	US 6361994	B1	20020326	US 2000-618301	20000718
PRAI	US 1995-497206	A1	19950630		
	US 1997-800965	A1	19970218		
	US 1998-79783P	P	19980327		
	US 1998-106170P	P	19981029		
	US 1998-183548	A2	19981030		
	US 1994-268969	A2	19940630		
	US 1999-277665	A1	19990326		

RE.CNT 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 7 OF 49 CAPLUS COPYRIGHT 2002 ACS
AN 2002:637552 CAPLUS
DN 137:179881
TI Flk1/KDR-based method for inhibiting angiogenesis, treating cancer, and increasing hematocrit levels
IN Kuo, Calvin; Mulligan, Richard
PA Children's Medical Center Corporation, USA; The Board of Trustees of the Leland Stanford Junior University
SO PCT Int. Appl., 62 pp.
CODEN: PIXXD2
DT Patent
LA English

6.1.8 SECTIONING OF NERVES

At specified time points, rats were euthanized by over-anesthesia with ether and their optic nerves were surgically removed, immersed in Tissue-Tek (Miles Inc., USA), and frozen in liquid nitrogen cooled in iso-pentane (BDH, UK). The nerves were then transferred to dry ice and stored at -70°C until sectioning. Longitudinal cryostat nerve sections (20 μm thick) were picked up onto gelatin-coated glass slides (four sections per slide) and frozen at -20°C until viewed or prepared for fluorescence staining.

6.1.9 DATA ANALYSIS OF T-CELLS IN NERVE SECTIONS

Nerves excised at various time periods after injury were prepared and sectioned. Hoechst-labeled nuclei or immunostained cells in each section were counted using the fluorescence microscope. For each time point five sections were counted, and the numbers were averaged.

6.1.10 IMMUNOLABELING OF NERVE SECTIONS

Longitudinal cryostat nerve sections (20 μm thick) were picked up onto gelatin glass slides and frozen until preparation for fluorescent staining. Sections were thawed and fixed in ethanol for 10 minutes at room temperature, washed twice with double-distilled water (ddH_2O), and incubated for 3 minutes in PBS containing 0.05% polyoxyethylene-sorbitan monolaurate (Tween-20; Sigma, USA). They were then incubated overnight at 4°C with a mouse monoclonal antibody directed against rat macrophages (ED1; 1:400; Serotec, UK) and antibody against rat glial fibrillary acidic protein (GFAP; 1:100; BioMakor), all diluted in PBS containing 3% FCS. Staining of T-cells was accomplished by incubating nerve sections for 1 hr at room temperature with a mouse monoclonal antibody directed against rat T-cell receptor (TCR) (1:100, Hunig *et al.*, *J. Exp. Med.*, 169:73, 1989), in PBS containing 3% FCS and 2% BSA. After three washes with PBS containing 0.05% Tween-20, the sections were incubated with goat anti-mouse $F(ab')_2$ conjugated to either

fluorescein isothiocyanate (FITC; BioMakor) or tetramethyl rhodamine isothiocyanate (TRITC; BioMakor) at a dilution of 1:100 and 1:50 respectively, for 1 hr at room temperature. They were then washed with PBS containing Tween-20 and treated with glycerol containing 1,4-diazobicyclo-(2,2,2) octane (Sigma), to inhibit quenching of fluorescence. The sections were viewed with a Zeiss Universal fluorescence microscope using filters that detect TRITC, FITC and Hoechst stains (Blaugrund *et al., Exp. Neurol.*, 118:105, 1992, Blaugrund *et al., Brain Res.*, 574:244, 1992).

6.2 RESULTS

6.2.1 ACCUMULATION OF ACTIVATED T-CELLS

T-cell clones primed to MBP (T_{MBP}) were activated with MBP for 2 days before being labeled with Hoechst stain and injected into animals i.p. at the time of injury. At 3, 7, 14 and 21 days after injury, the nerves were excised, cryosectioned and analyzed microscopically for the presence of labeled T-cells.

T_{MBP} cells were detected in the injured optic nerves at day 3 and accumulated until a peak at day 14 (Fig. 1). Large clusters of T_{MBP} cells were observed at the injury site and fewer individual cells were seen proximal and distal (Fig. 2). Four weeks after injury, labeled T-cells were still detectable in the degenerating optic nerves. No T-cells were found in the non-injured optic nerves (Fig. 3), non-injured sciatic nerve or injured sciatic nerve at any time after injury. Labeled T-cells were occasionally found in capillaries and in connective tissue but were not concentrated or localized into any specific areas. T-cells that were not prestimulated with antigen did not accumulate in any of the nerves, including damaged nerves.

The accumulation of T_{MBP} cells in injured CNS, but not in injured PNS, suggests that there might be some specific interaction between the primed T-cells and the CNS tissue from which the MBP antigen was originally derived. To determine whether the injured CNS interacted with T-cells in

general, or specifically with T-cells primed with a CNS antigen, the previous experiments were repeated using a clone that responds to chick ovalbumin (T_{OVA}). Rats were injected with a labeled T_{OVA} clone prestimulated with ovalbumin (OVA) using the same protocol as with the T_{MBP} cells. The labeled T_{OVA} cells accumulated in injured optic nerve, and the pattern of accumulation was similar to that of the T_{MBP} cells. Labeled T_{OVA} and T_{MBP} cells were counted in longitudinal sections of optic nerve prepared, 3, 7, 14 and 21 days after injury. No significant different was observed in numbers of T_{MBP} and T_{OVA} cells in injured optic nerve (Fig. 4), indicating that antigen specificity has little to do with the accumulation of T-cells in CNS injury sites. T_{MBP} cells were detectable slightly earlier than T_{OVA} cells in the optic nerve injury site, and antigen specificity may play a role in this but is not sufficient to explain the large accumulation of T_{OVA} cells in the site of injury.

Fig. 5 shows accumulation of T-cells measured immunocytochemically using antibodies to T-cell receptors. This detection technique rules out the possibility that the observed labeling is due to phagocytic cells which had phagocytized the pre-labeled T-cells shown in Fig. 1. The graph shows a striking elevation in T-cell accumulation following injury, regardless of whether the systematically injected T-cells are specific to a self-epitope (MBP) or to a non-self epitope (OVA).

Fig. 6 shows that accumulation of T-cells is dependent on the lesion and not the breakdown of the blood-brain barrier. T-cells specific to either MBP or OVA were injected 2 weeks after injury and their accumulation analyzed a week later, namely 21 days following the primary lesion. Their accumulation was compared to that of T-cells injected immediately after injury and detected either 7 or 21 days later. It appears that the time elapsed between the injury and the injection of T-cells, which is a factor in the sealing of the blood-brain barrier, is not a factor in the T-cell accumulation.

7. EXAMPLE: USES OF ACTIVATED T-CELLS AND NS-SPECIFIC ATCs

7.1 MATERIALS AND METHODS

Animals, proteins used for T-cell stimulation, media, crush injury of rat optic nerve, sectioning of nerves, immunolabeling of nerve sections, and data analysis of T-cells in nerve sections are described in Section 6, *supra*.

7.1.1 ESTABLISHMENT OF T-CELL LINES WITH ACTIVE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE)

MBP and OVA were dissolved in PBS (1 mg/ml) and emulsified with an equal volume of Incomplete Freund's Adjuvant (IFA) supplemented with 4 mg/ml *M. tuberculosis*. Rats were immunized subcutaneously in the hind footpads with 0.1 ml of the emulsion. At day 9 (1-3 days before clinical onset of disease), animals were euthanized and draining lymph nodes were surgically removed and dissociated under sterile conditions. The cells were washed and placed in proliferation medium with irradiated thymocytes (2000 rads) and either 10 μg/ml of MBP, OVA or *M. tuberculosis* for 3 days. Cells were then washed and placed in propagation medium for 5 to 10 days at which time they were re-exposed to irradiated thymocytes and peptides in proliferation medium. T-cell lines were expanded by re-exposure and propagation and tested for specificity in an antigen specific T cell proliferation assay. Lines were expanded and stocks were frozen in liquid nitrogen. The cells were thawed and stimulated once before being used in experiments.

7.1.2 PASSIVE TRANSFER OF T-CELL LINES

T-cell lines were activated by restimulation *in vitro* with their own antigen (10 μg/ml) in proliferation medium. After incubation for 48-72 hrs at 37°C 90% relative humidity and 7.5% CO_2, the cells were washed. Viable cells were isolated on Percoll and suspended in PBS. Animals were injected with 10 x 10^6 cells/ml i.p. Control animals were injected with 1 ml PBS i.p.

7.1.3 CRUSH INJURY OF RAT SCIATIC NERVE

Under deep anesthesia as described in Section 6.1.5, the sciatic nerve was exposed and a similar crush injury was inflicted. At the end of the operation the skin was sutured.

7.1.4 RETROGRADE LABELING OF RGCs

The optic nerve was exposed, without damaging the retinal blood supply. Solid crystals of the dye, 4-(4-(didecylamino)styryl)-n-methyl-pyridinium iodide (4-Di-10-Asp) (Molecular Probes, Europe BV), were deposited 1-2 mm from the distal border of the injury site. Non-injured optic nerves were similarly labeled at approximately the same distance from the globe. Five days after dye application, the retinas were excised under deep anesthesia, flat mounted in 4% paraformaldehyde solution, and labeled retinal ganglion cells (RGCs) were counted by fluorescence microscopy.

7.1.5 ASSESSMENT OF EFFECTS OF INJECTED T-CELLS

The effect of injected T-cells on the numbers of surviving optic nerve fibers was monitored by retrograde labeling of RGCs (see above) immediately after injury in order to assess primary degeneration and two weeks later in order to assess secondary degeneration. Five days after dye (4-Di-10-Asp) application, the retinas were excised, whole mounted and their RGCs were counted. The counting was done in five randomly selected fields in each retina (all located at approximately the same distance from the optic disc). In all cases the dye was applied 2 ml distally to the site of the prior insert. Using this lengthening approach, only those RGCs whose axons were still viable could be labeled. The numbers of RGCs in each group of injured nerves treated with PBS only were injected with T_{MBP} or T_{OVA} cells. Results were expressed as percentage of axons, out of those which survived the primary insult (42% the axons remained after the primary insult).

7.1.6 CLINICAL EVALUATION OF EAE

Clinical disease was scored every 1 to 2 days according to the following neurological scale: 0, no abnormality; 1, tail atony; 2, hind limb paralysis; 3, paralysis extending to thoracic spine; 4, front limb paralysis; 5, moribound state.

7.2 RESULTS

7.2.1 ACCUMULATION OF NS-SPECIFIC ATCS

The injured optic nerve was analyzed for T-cell accumulation. As shown in Fig. 7, in the uninjured optic nerves of control rats injected with phosphate-buffered saline (PBS) no T-cells could be detected. Small but significant numbers of T-cells were observed in the uninjured optic nerves of rats injected with anti-MBP T-cells (primed against a peptide comprising amino acids 51-70 of MBP "*P51-70*" known to be capable of inducing experimental autoimmune encephalomyelitis (EAE) under these experimental conditions), but not of rats injected with anti-OVA T-cells. Crush injury of the optic nerve was accompanied by a small but significant accumulation of endogenous T-cells, possibly reflecting a response to self antigens triggered by the injury. In the injured optic nerves, T-cell accumulation was significantly increased by 5- to 6-fold) in rats injected with anti-OVA, anti-hsp60, or anti-MBP T-cells. These observations confirmed our previous finding that axonal injury in the CNS is accompanied by the accumulation of endogenous T-cells and that this accumulation is augmented by systemic injection of activated T-cells irrespective of their antigenic specificity.

7.2.2 PROTECTION OF SECONDARY NERVE DEGENERATION BY MBP SPECIFIC T-CELLS

The course of secondary degeneration as a result of the injected T-cells was then examined. Previous studies have shown that a time lapse of 2 weeks between a crush injury (of similar severity to the present one) and dye application is optimal for demonstrating differences (in terms of the

numbers of still-viable, i.e. labeled, neurons) in degeneration with and without neuroprotection. As shown in Fig. 8, in retinas of injured nerves that were subjected to dye application 2 weeks after injury and excised a week later, the number of labeled ganglion cells (reflecting still-viable axons) was about 2.5-fold greater in animals injected at the time of injury, with T-cells specific to MBP (primed against P51-70) than with PBS. In contrast, labeled ganglion cells in the retinas of rats injected with anti-OVA or anti-hsp60 T-cells were not significantly more numerous than in the retinas of rats injected with PBS. Fig. 9 represents micrographs of retrogradeably labeled retinas of injured optic nerves of rats injected with PBS, anti-hsp60 T-cells, or anti-MBP T-cells.

Since only the anti-MBP T-cells showed a neuroprotective effect, and as hsp60, like MBP, is a self antigen which is expressed in injured tissues including EAE lesions, it was interesting to find out whether the protective effect of the anti-MBP T-cells is *a function* of their *aggressiveness in causing* an autoimmune disease. If so, this would explain the lack of a protective effect by hsp60, which is also a self antigen, but, unlike MBP, is not restricted to the CNS and the T-cells specific to it do not cause a disease. To explore the possibility of a connection between the observed neuroprotective effect and autoimmune disease, the effect of T-cells generated against an epitope in MBP (P51-70) that does not cause an autoimmune disease was examined. As shown in Fig. 10, the neuroprotective effect of these non-aggressive anti-MBP T-cells was similar to that of the anti-MBP T-cells that cause autoimmune diseases. It thus seems that the observed beneficial effect of the T-cells on secondary degeneration is not common to all self antigens, but in this study is restricted to NS-specific antigens. Furthermore, T-cells activated with a fragment of a NS-specific antigen that does not cause autoimmune disease were substantially as effective in inhibiting secondary

degeneration as T-cells activated with full-length NS-specific antigen that does cause autoimmune disease.

7.2.3 CLINICAL SEVERITY OF EAE

Animals were injected i.p. with 10^7 T_{MBP} cells with or without concurrent optic nerve crash injury. The clinical course of the rats injected with the T_{MBP} cells was evaluated according to the neurological paralysis scale. Each group contained 5-9 rats. As can be seen in Fig. 11, the course and degree of the EAE was not affected by whether or not the rats had been subjected to an optic nerve crash.

7.2.4 SURVIVAL OF RGCS IN NON-INJURED NERVES

Animals were injected i.p. with 10^7 T_{MBP} cells or PBS. Two weeks later, 4-Di-10-Asp was applied to the optic nerves. After five days the retinas were excised and flat mounted. Labeled RGCs from five fields, in each retina were counted and their average number per mm^2 was calculated.

As can be seen in Fig. 12, there is no difference in the number of surviving RGCs in non-injured optic nerves of rats injected with anti-MBP T-cells compared to rats injected with PBS.

8. EXAMPLE: NEUROPROTECTIVE EFFECTS OF NS-SPECIFIC ANTIGEN

8.1 MATERIALS AND METHODS

Animals, crush injury of rat optic nerve, and retrograde labeling are described above in Sections 6 and 7. A peptide based on amino acids 35-55 of myelin/oligodendrocyte glycoprotein (MOG p35-55) was chemically synthesized at the Weizmann Institute, Israel.

8.1.1 INHIBITION OF SECONDARY DEGENERATION

Rats were injected intradermally in the footpads with MOG p35-55 (50 μg/animal) and IFA, or PBS ten days prior to optic nerve crush injury. Retinal ganglion cells were assessed two weeks after injury using retrograde labeling as

described above. The number of RGCs in rats injected with PBS or MOG p35-55 was expressed as a percentage of the total number of neurons in rats injected with MOG p35-55 in the absence of crush injury.

8.2 RESULTS

As shown in Fig. 13, the number of labeled ganglion cells (indicating viable axons) was about 12.5 fold greater in animals injected with MOG p35-55 compared to animals receiving PBS.

9. EXAMPLE: NEUROPROTECTIVE EFFECTS OF MBP ADMINISTERED ORALLY

9.1 MATERIALS AND METHODS

Animals, crush injury of rat optic nerve, and retrograde labeling of RGCs are described above in Sections 6 and 7.

9.1.1 INHIBITION OF SECONDARY DEGENERATION

Bovine MBP (Sigma, Israel) (1 mg/dose) was administered to rats by gavage using a blunt needle. MBP was administered 5 times, every third day, beginning 2 weeks prior to optic nerve crush injury. The number of RGCs in treated animals was expressed as a percentage of the total number of neurons in animals subjected to optic nerve crush injury but which did not receive MBP.

9.2 RESULTS

As shown in Fig. 14, the number of labeled RGCs was about 1.3 fold greater in animals treated with MBP compared to untreated animals.

10. DISCUSSION

The results of the experiments described in Sections 6 and 7 show that activated T-cells accumulate at a site of injury in the CNS. Furthermore, the results also demonstrate that the accumulation of T-cells at the site of injury is a non-specific process, *i.e.*, T-cells which accumulated at the

site of injury included both T-cells which are activated by exposure to an antigen present at the site of injury as well as T-cells which are activated by an antigen not normally present in the individual.

The results of experiments described in Section 7 demonstrate that the beneficial effects of T-cells in ameliorating damage due to injury in the CNS are associated with a NS-specific self-antigen as illustrated by MBP. More specifically, the administration of non-recombinant T-cells which were activated by exposure to an antigen which causes autoimmune disease (T_{MBP}), rather than aggravating the injury, led to a significant degree of protection from secondary degeneration. Thus, activating T-cells by exposure to a fragment of a NS-specific antigen was beneficial in limiting the spread of injury in the CNS. The present findings show that secondary degeneration can be inhibited by the transfer into the individual of T-cells which recognize a NS-specific self antigen which is present at a site of injury.

In addition, the studies described in Sections 8 and 9 show that activation of T-cells by administering an immunogenic antigen (*e.g.* MBP) or immunogenic epitope of an antigen (*e.g.* MOG p35-55), may be used for preventing or inhibiting secondary CNS degeneration following injury.

The present application claims priority benefits of Israeli patent application IL 124550, filed May 19, 1998, the disclosure of which is incorporated herein by reference in its entirety.

The present invention is not to be limited in scope by the exemplified embodiments, which are intended as illustrations of single aspects of the invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications cited herein are incorporated by reference in their entirety.

WHAT IS CLAIMED IS:

1. A method for delivering a therapeutic or detectable substance to a site of injury or disease of the central nervous system (CNS), comprising administering activated T-cells that do not recognize a nervous system specific (NS-specific) antigen in which the activated T-cells contain or express a therapeutic or detectable substance to a mammal.

2. The method according to claim 1 in which said activated T-cells are generated by exposing T-cells to a cognate non-self antigen or a mitogen.

3. The method according to claim 1 in which said activated T-cells endogenously produce said substance.

4. The method according to claim 1 in which said activated T-cells are T-cells that have been genetically-engineered to express said therapeutic substance or to express an enzyme which catalyzes production of said therapeutic substance or to express a regulatory product, that induces production of said therapeutic substance.

5. The method according to claim 1 in which said injury comprises blunt trauma, penetrating trauma, hemorrhagic stroke, or ischemic stroke.

6. The method according to claim 1 in which said disease is selected from the group consisting of Alzheimer's disease, Parkinson's disease, multiple sclerosis, glaucoma, Huntington's disease, amyotrophic lateral sclerosis, and Creutzfeldt-Jakob disease.

7. A recombinant T-cell comprising a promoter operably linked to a nucleotide sequence that encodes a protein that ameliorates the effects of an injury or disease

of the central nervous system (CNS) wherein the recombinant T-cells do not recognize a nervous system specific (NS-specific) antigen.

8. A method for delivering a substance to a site of injury or disease of the CNS, comprising administering to a mammal the recombinant T-cell of claim 7.

9. The method according to claim 1 or 8 in which said mammal is a human.

10. The method according to claim 8 in which said recombinant T-cell is produced using an autologous T-cell.

11. A method for detecting a site of injury or disease of the central nervous system (CNS) in a mammal, comprising:

a) administering to a mammal an effective amount of labeled activated T-cells that do not recognize a nervous system specific (NS-specific) antigen; and

b) detecting in the mammal the labeled activated T-cells that have accumulated at said site of injury or disease, in which step (b) is performed after an interval sufficient to permit said labeled activated T-cells administered in step (a) to accumulate at said site of injury or disease.

12. The method according to claim 11 in which the mammal is a human.

13. The method according to claim 1, 8 or 11 in which the activated T-cells recognize a non-self antigen.

14. The method according to claim 11 in which the detectable substance is detected *in vivo*.

15. The method of claim 11 in which the labeled activated T-cells are labeled with a radioisotope or a contrast agent.

16. The method of claim 1, 8 or 11 in which the labeled activated T-cells are administered intravenously or intraperitoneally.

17. A method for preventing or inhibiting degeneration in the central nervous system (CNS) of a mammal comprising administering non-recombinant, NS-specific antiself activated T-cells in which said activated T-cells ameliorate the effects of injury or disease.

18. A method for preventing or inhibiting degeneration in the central nervous system (CNS) of a mammal comprising administering an effective amount of a NS-specific antigen.

19. The method according to claim 18 in which said NS-specific antigen activates T-cells *in vivo* resulting in a population of T-cells that accumulate at a site of injury or disease of the CNS.

20. The method according to claim 18 in which said NS-specific antigen is administered intravenously, intraperitoneally, orally, nasally or buccally.

21. The method according to claim 2 in which said T-cells are autologous.

22. A pharmaceutical composition comprising isolated, activated T-cells that do not recognize a nervous system specific (NS-specific) antigen in which the activated T-cells contain an exogenously added detectable substance or contain a therapeutic substance or express a recombinant

substance having a therapeutic effect when administered *in vivo* to a mammal; and a pharmaceutically acceptable carrier.

23. A pharmaceutical composition comprising a therapeutically effective amount of an isolated, NS-specific antigen; and a pharmaceutically acceptable carrier.

24. Use of an active ingredient comprising non-recombinant NS-specific antiself T-cells or a NS-specific antigen, for the preparation of a pharmaceutical composition for the treatment of a human condition or disease of the CNS.

25. Use according to claim 24 in which the untreated human condition or disease results in central nervous system degeneration.

26. Use of an active ingredient comprising recombinant, activated T-cells that do not recognize a nervous system specific (NS-specific) antigen, for the preparation of a pharmaceutical composition for the treatment of a human condition or disease of the CNS.

AMENDED CLAIMS

[received by the International Bureau on 21 December 1998 (21.12.98); original claims 1-26 replaced by new claims 1-32 (5 pages)]

1. A method for delivering a therapeutic or detectable substance to a site of injury or disease of the central nervous system (CNS), comprising administering to a mammal activated T-cells that do not recognize a nervous system specific (NS-specific) antigen in which the activated T-cells contain or express a therapeutic or detectable substance.

2. The method according to claim 1 in which said activated T-cells are generated by exposing T-cells to a non-self antigen or a mitogen.

3. The method according to claim 1 in which said activated T-cells endogenously produce said therapeutic substance.

4. The method according to claim 1 in which said activated T-cells are T-cells that have been genetically-engineered to express said therapeutic substance or to express an enzyme which catalyzes production of said therapeutic substance or to express a regulatory product, that induces production of said therapeutic substance.

5. The method according to claim 4 in which the genetically engineered T-cells express said therapeutic substance or enzyme or regulatory product under the control of necessary elements for transcription or translation continuously or after induction.

6. The method according to claim 1 in which said injury comprises blunt trauma, penetrating trauma, trauma sustained during a neurosurgical operation, hemorrhagic stroke, or ischemic stroke.

7. The method according to claim1 in which said disease is selected from the group consisting of Alzheimer's disease, Parkinson's disease, multiple sclerosis, glaucoma, optic nerve injury accompanying optic neuropathy, Huntington's disease, amyotrophic lateral sclerosis, and Creutzfeldt-Jakob disease.

8. A recombinant T-cell comprising a promoter operably linked to a nucleotide sequence that encodes a protein that ameliorates the effects of an injury or disease of the central nervous system (CNS) wherein the recombinant T-cells do not recognize a nervous system specific (NS-specific) antigen.

9. The recombinant T-cell according to claim 8 in which the protein is a therapeutic substance or an enzyme which catalyzes production of said therapeutic substance or a regulatory product, that induces production of a therapeutic substance.

10. A method for delivering a substance to a site of injury or disease of the CNS, comprising administering to a mammal the recombinant T-cell of claim 8.

11. The method according to claim 1 or 10 in which said mammal is a human.

12. The method according to claim 10 in which said recombinant T-cell is produced using an autologous T-cell.

13. A method for detecting a site of injury or disease of the central nervous system (CNS) in a mammal, comprising:

a) administering to a mammal an effective amount of labeled activated T-cells that do not recognize a nervous system specific (NS-specific) antigen; and

b) detecting in the mammal the labeled activated T-cells that have accumulated at said site of injury or disease, in which step (b) is performed after an interval sufficient to permit said labeled activated T-cells administered in step (a) to accumulate at said site of injury or disease.

14. The method according to claim 13 in which the mammal is a human.

15. The method according to claim 1, 10 or 13 in which the activated T-cells recognize a non-self antigen.

16. The method of claim 13 in which the labeled activated T-cells are labeled with a radioisotope, a contrast agent or fluorescence-emitting metal.

17. The method of claim 1, 10 or 13 in which the activated T-cells are administered intravenously, intraperitoneally, intramuscularly or subcutaneously.

18. The method according to claim 2 in which said T-cells are autologous.

19. A pharmaceutical composition comprising isolated, activated T-cells that do not recognize a nervous system specific (NS-specific) antigen for use to deliver a therapeutic or detectable substance in which the activated T-cells contain a therapeutic substance or express a recombinant substance having a therapeutic effect or contain an exogenously added detectable substance when administered *in vivo* to a mammal; and a pharmaceutically acceptable carrier.

20. The pharmaceutical composition according to claim 19 in which said activated T-cells are generated by exposing T-cells to a non-self antigen or a mitogen.

21. The pharmaceutical composition according to claim 19 in which said activated T-cells endogenously produce said therapeutic substance.

22. The pharmaceutical composition according to claim 19 in which said activated T-cells are T-cells that have been genetically-engineered to express said therapeutic substance or to express an enzyme which catalyzes production of said therapeutic substance or a regulatory product, that induces production of said therapeutic substance.

23. The pharmaceutical composition according to claim 19 in which said activated T-cells express said therapeutic substance or enzyme or regulatory product under the control of necessary elements for transcription or translation continuously or after induction.

24. The pharmaceutical composition according to claim 19 in which said activated T-cells are used to treat injury comprising blunt trauma, penetrating trauma, penetrating trauma, trauma sustained during a neurosurgical operation, hemorrhagic stroke, or ischemic stroke.

25. The pharmaceutical composition according to claim 19 in which said activated T-Cells are used to treat disease selected from the group consisting of Alzheimer's disease, Parkinson's disease, multiple sclerosis, glaucoma, optic nerve injury accompanying optic neuropathy, Huntington's disease, amyotrophic lateral sclerosis, and Creutzfeldt-Jakob disease.

26. Use of an active ingredient comprising activated T-cells that do not recognize a nervous system specific (NS-specific) antigen, for the preparation of a pharmaceutical composition for the treatment or detection of a human condition or disease of the CNS.

27. The use according to claim 26 in which said activated T-cells are generated by exposing T-cells to a non-self antigen or a mitogen.

28. The use according to claim 26 in which said activated T-cells endogenously product said therapeutic substance.

29. The use according to claim 26 in which said activated T-cells are T-cells that have been genetically-engineered to express said therapeutic substance or to express an enzyme which catalyzes production of said therapeutic substance or to express a regulatory product, that induces production of said therapeutic substance.

30. The use according to claims 26 in which said activated T-cells express said therapeutic substance or enzyme or regulatory product under the control of necessary elements for transcription or translation continuously or after induction.

31. The use according to claim 26 in which said activated T-cells are used to treat injury comprising blunt trauma, penetrating trauma, trauma sustained during a neurosurgical operation, hemorrhagic stroke, or ischemic stroke.

32. The use according to claim 26 in which said activated T-cells are used to treat disease selected from the group consisting of Alzheimer's disease, Parkinson's disease, multiple sclerosis, glaucoma, optic nerve injury accompanying optic neuropathy, Huntington's disease, amyotrophic lateral sclerosis, and Creutzfeldt-Jakob disease.

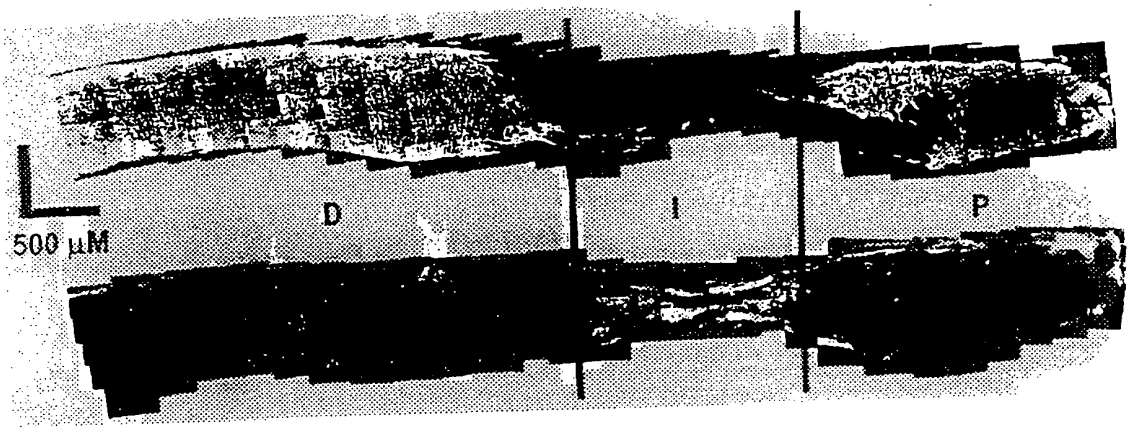

FIG. 1

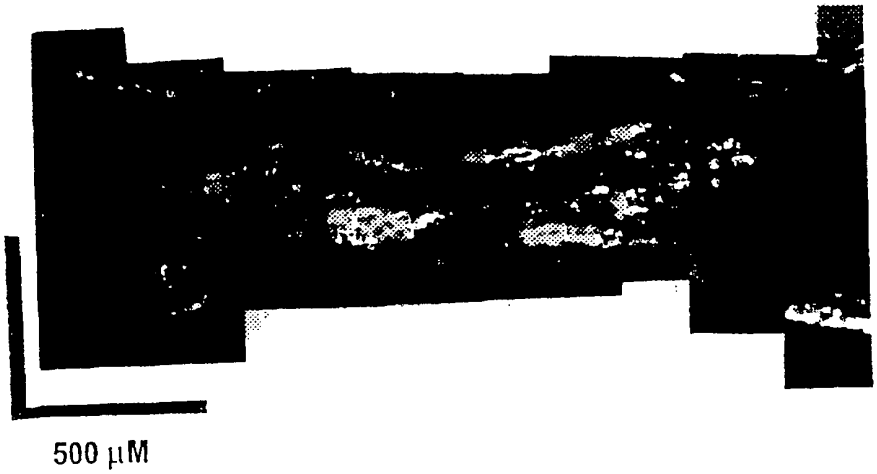

FIG. 2

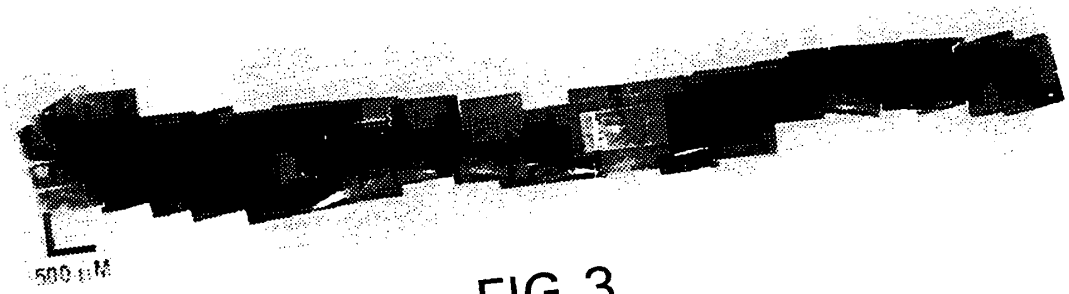

FIG.3

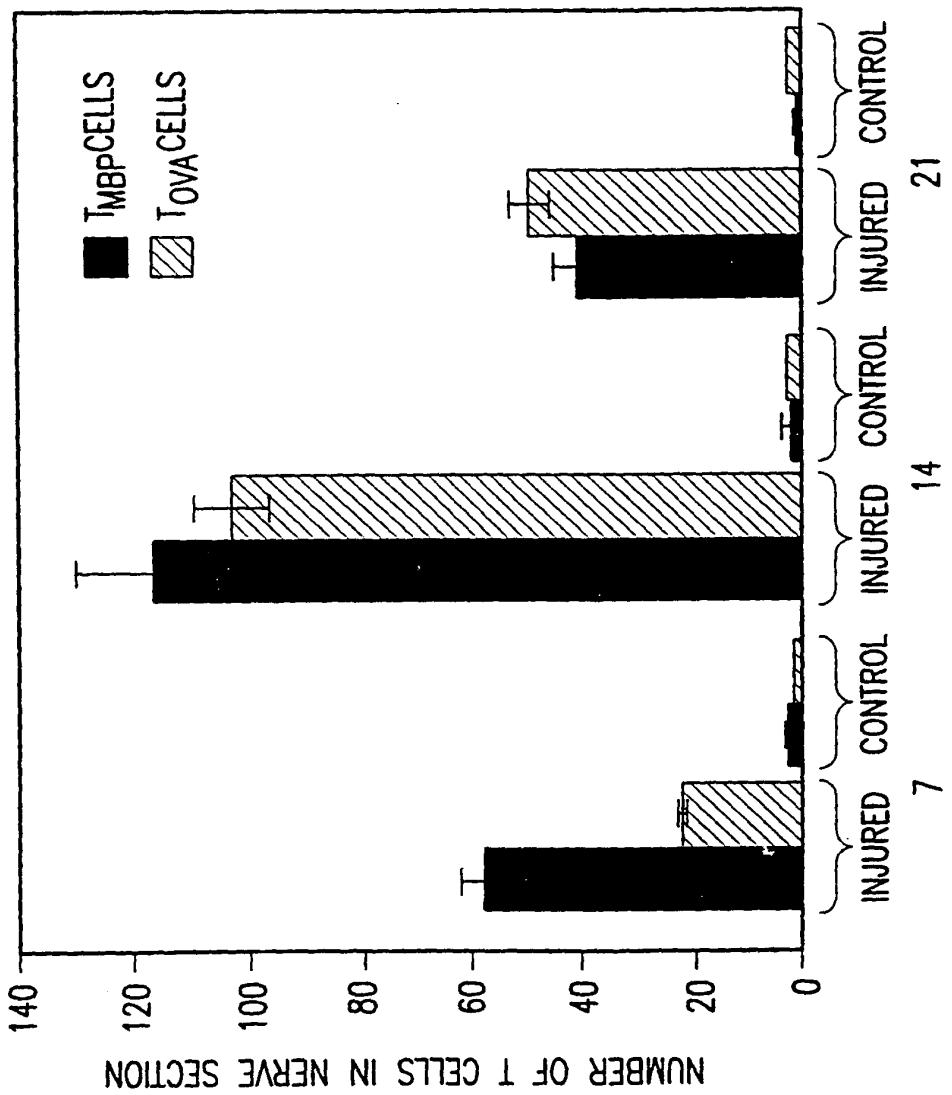

FIG.4

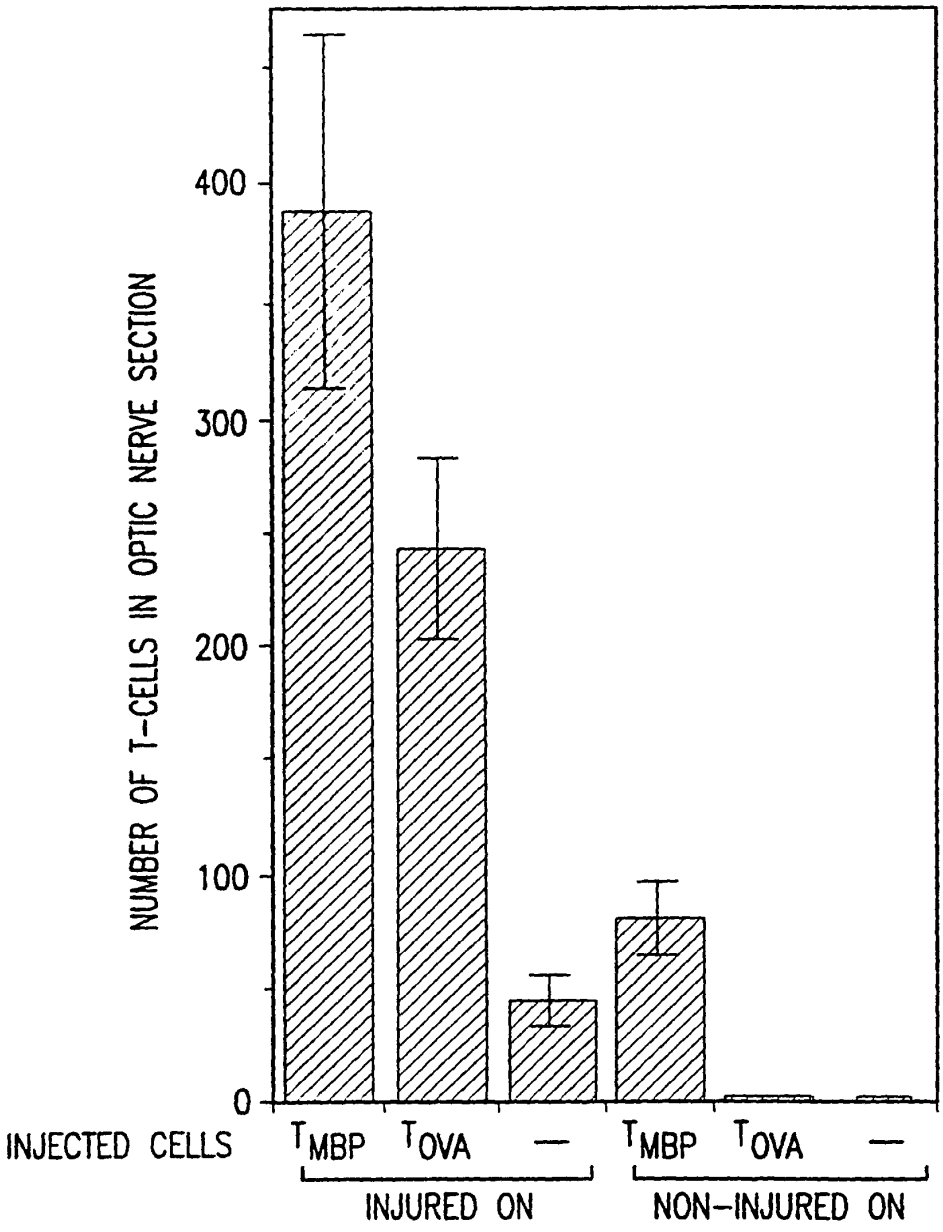

FIG.5

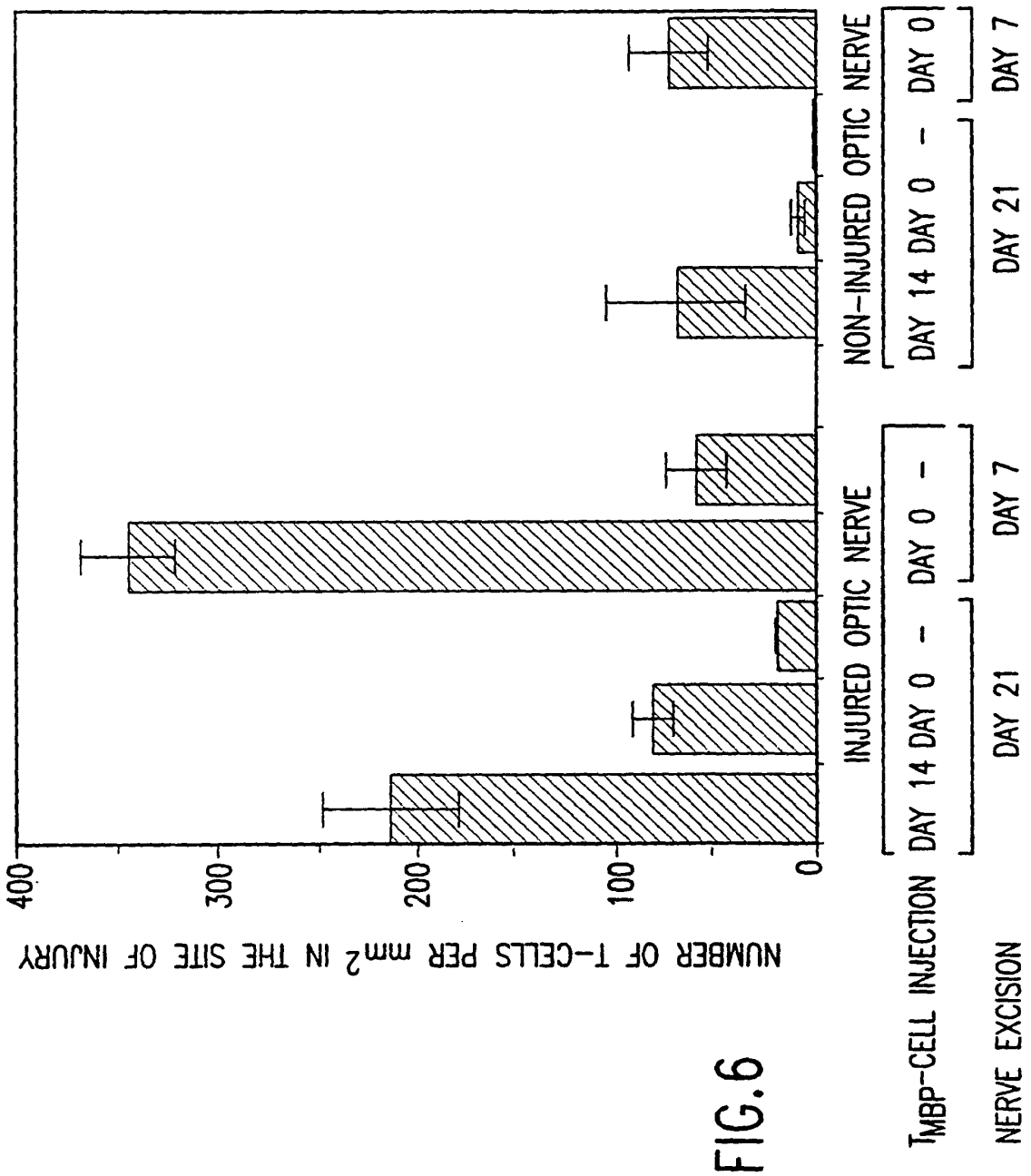

FIG.6

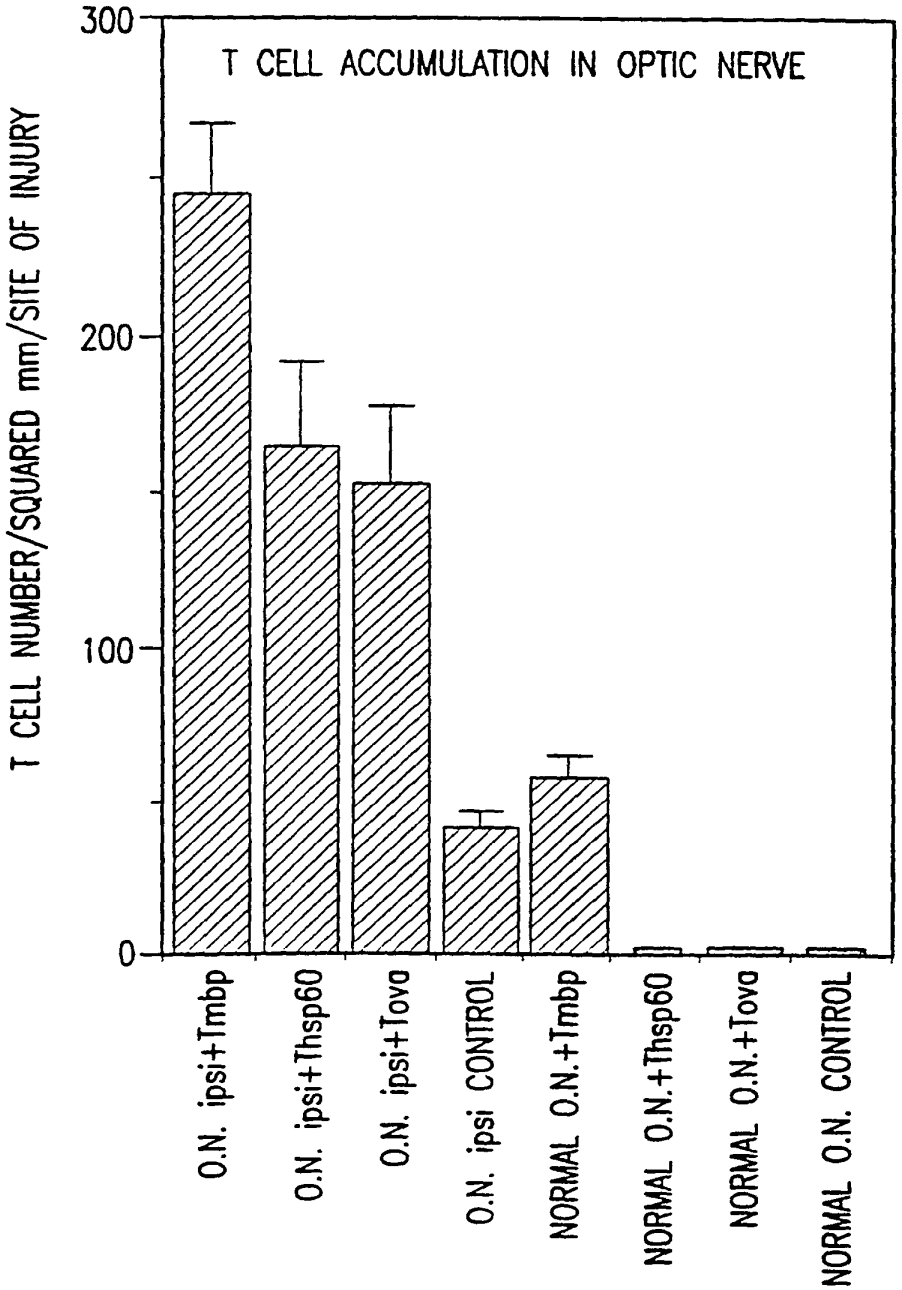

FIG.7

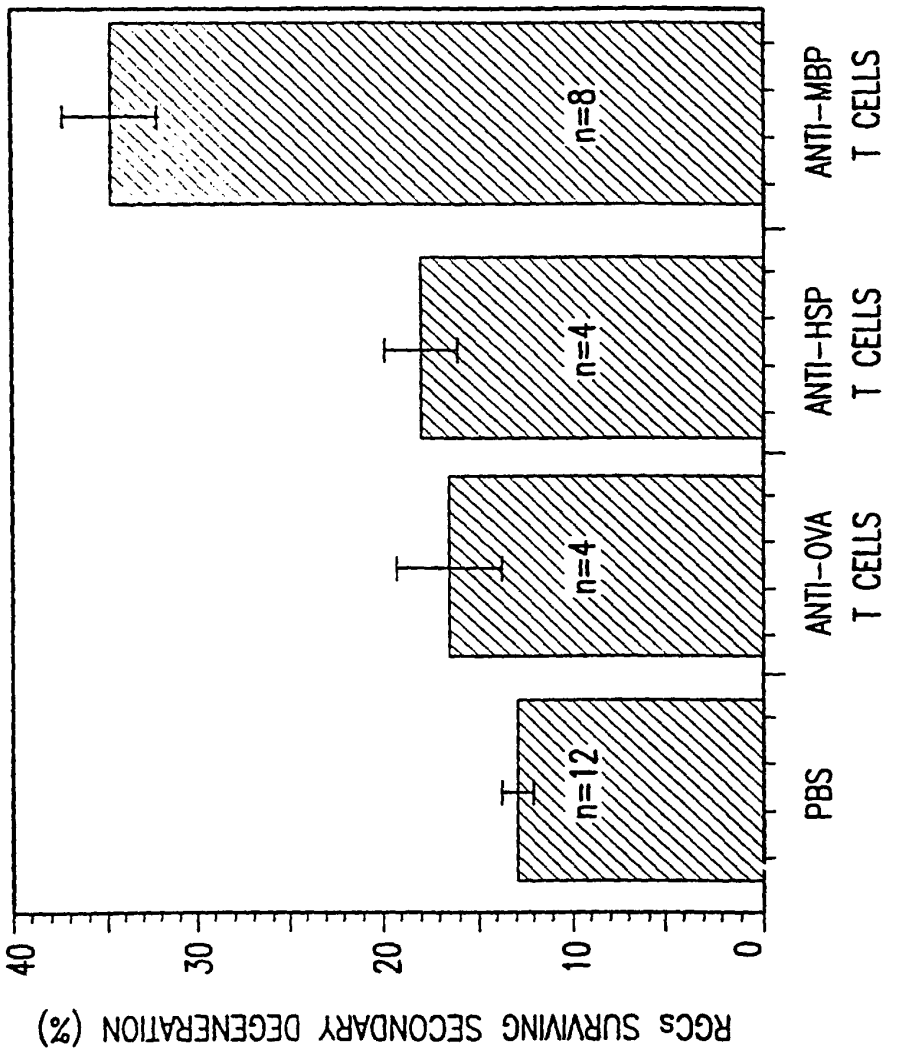

FIG.8

FIG.9C

FIG.9B

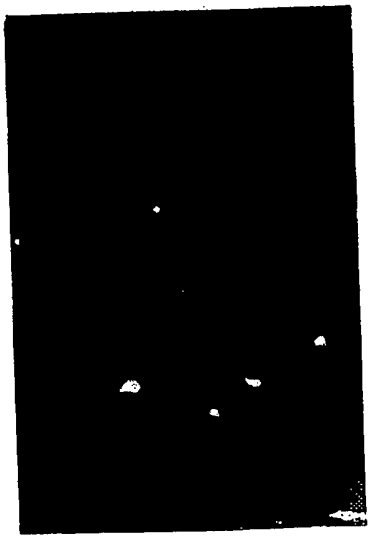

FIG.9A

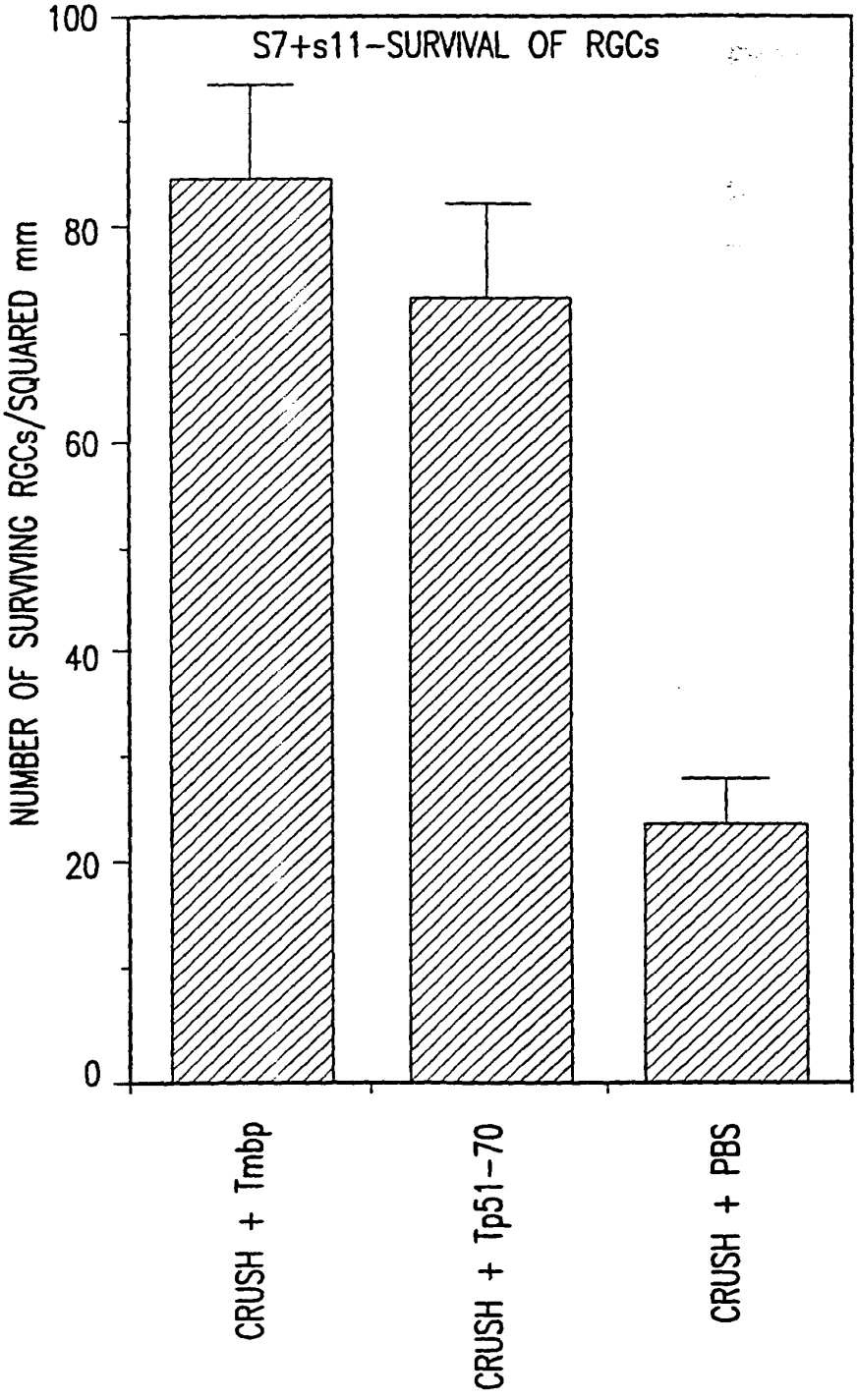

FIG.10

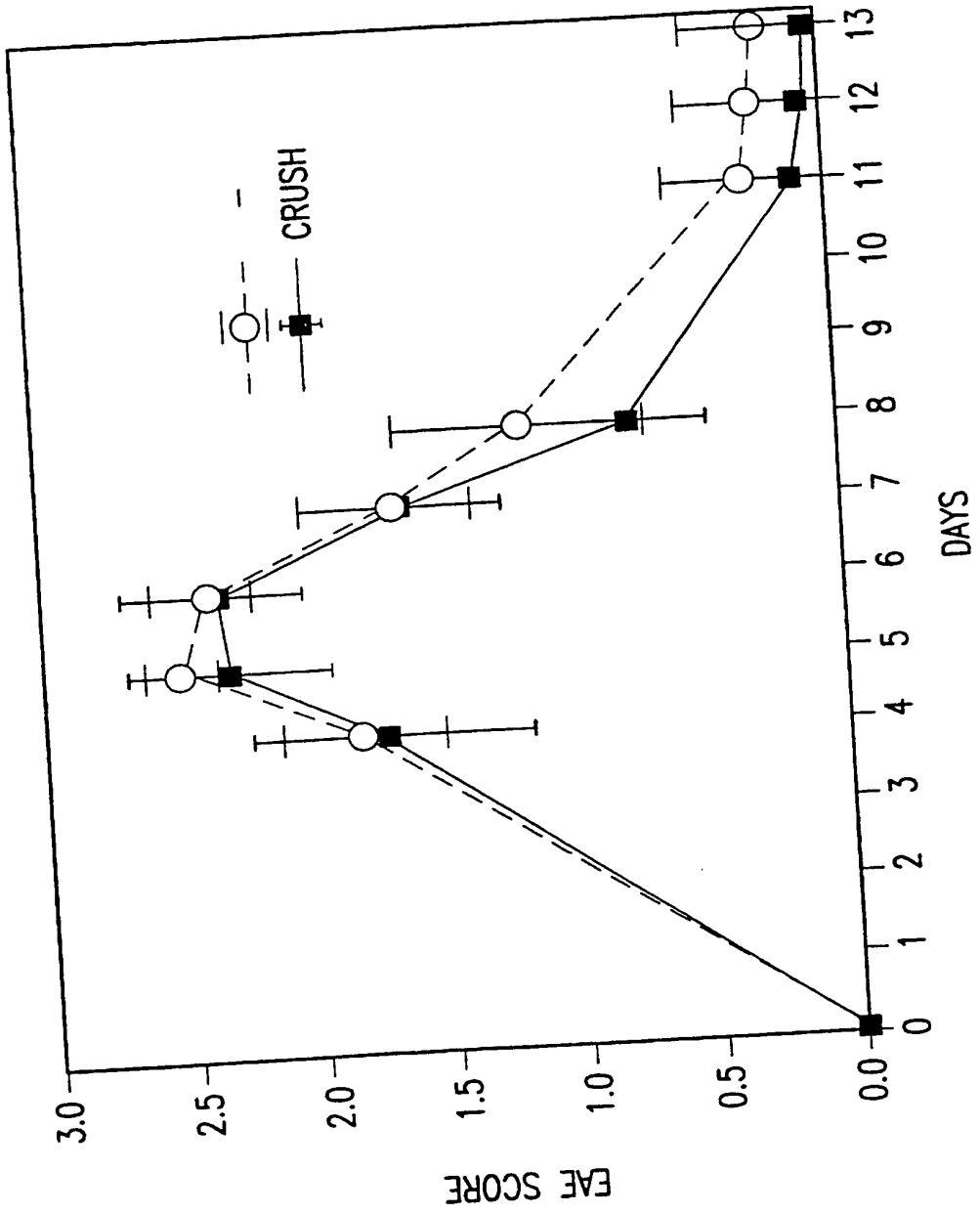

FIG.11

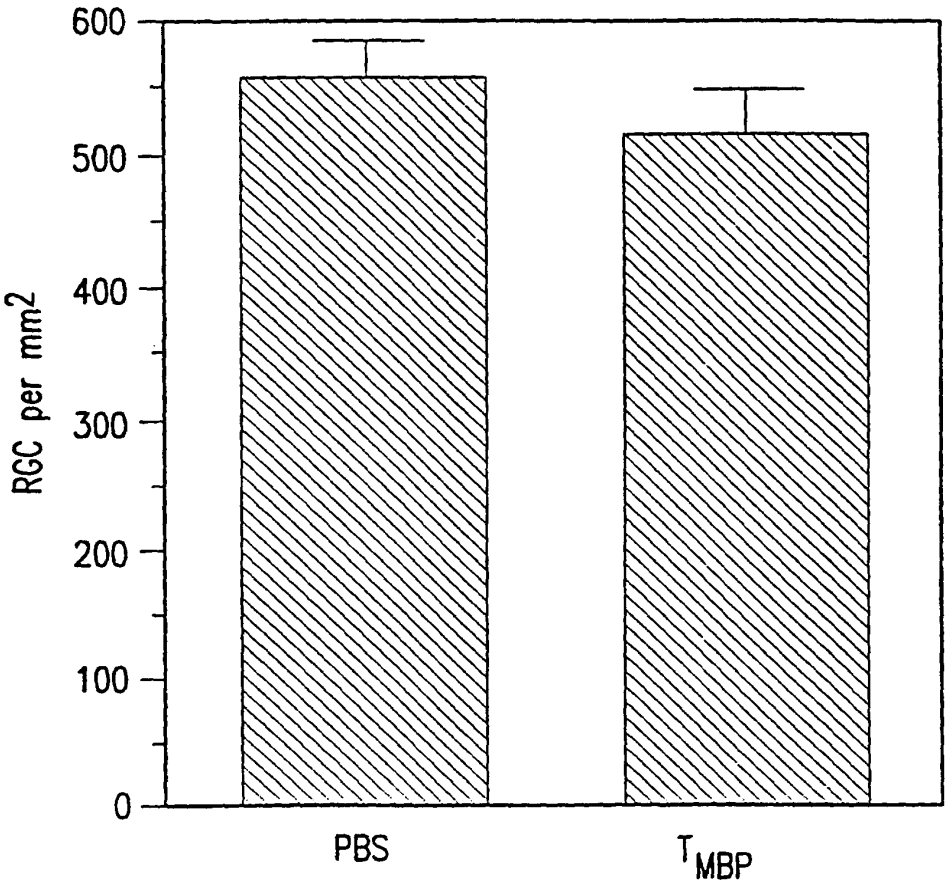

FIG.12

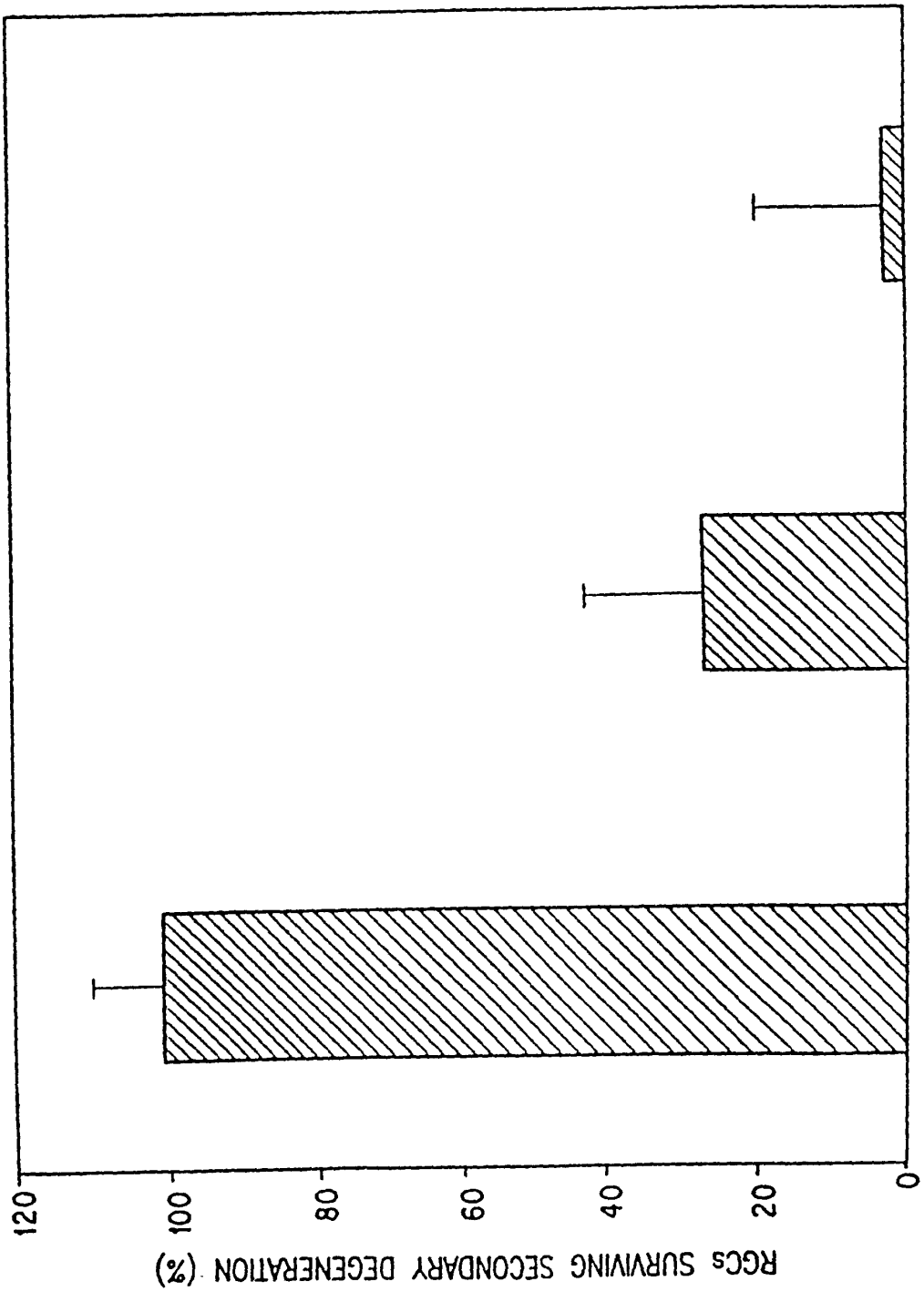

FIG. 13

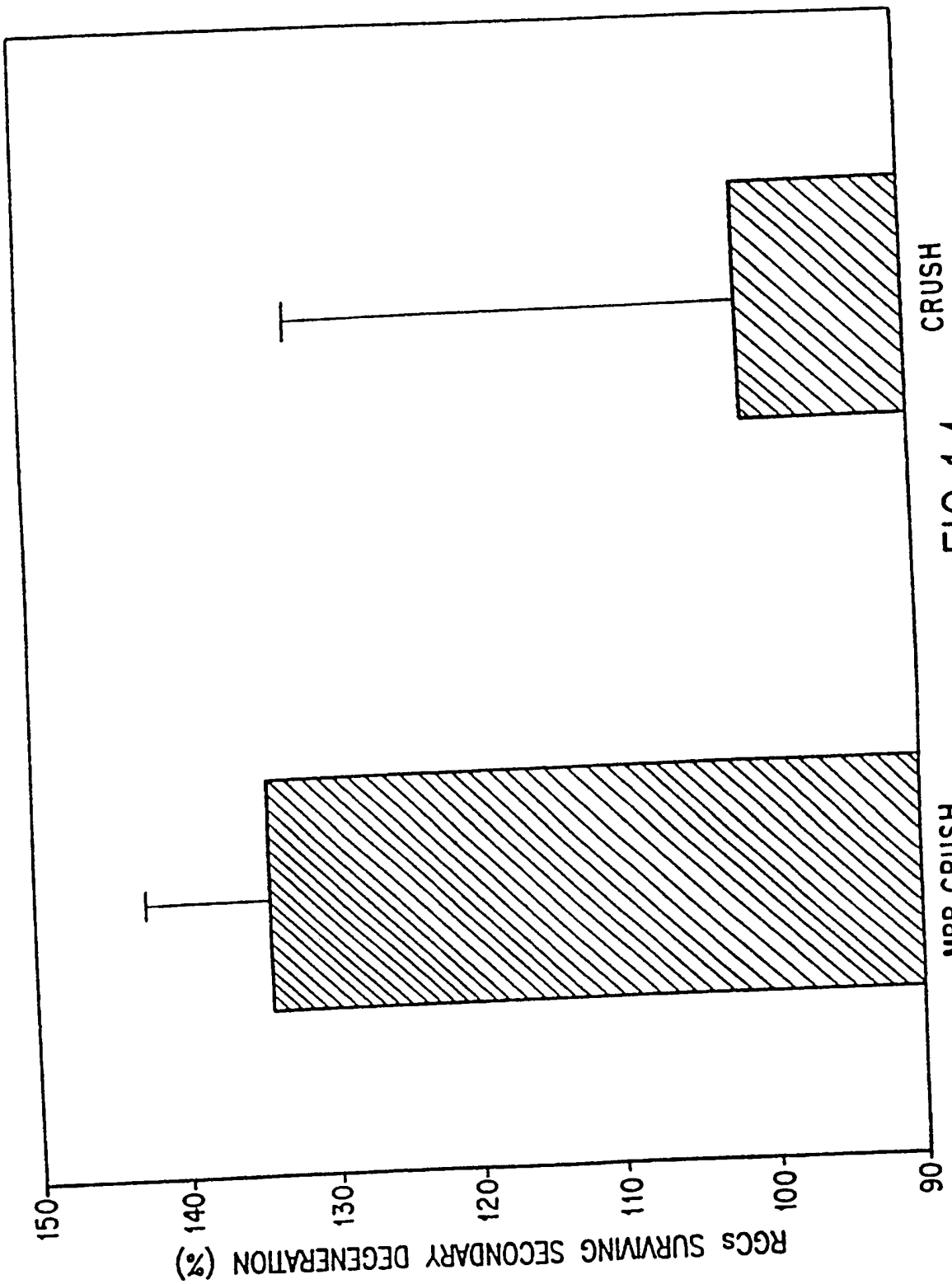

FIG. 14